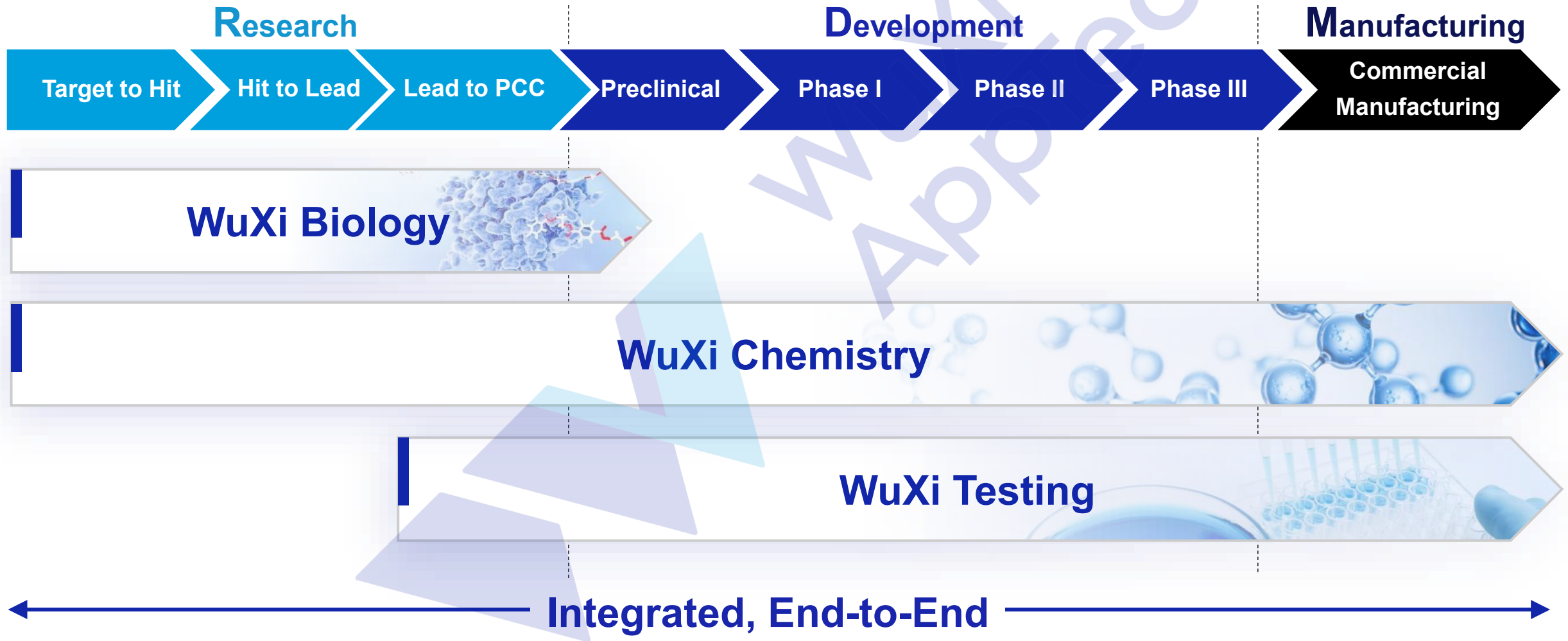


From Library to Lead: Unlocking Peptide Therapeutics with DNA- Encoded Libraries and Display Platforms

*Early Discovery Platform
In Vitro Biology Unit, WuXi Biology*

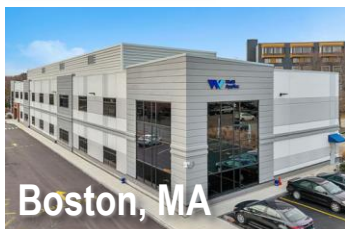


Integrated End-to-End CRDMO Enabling Platform



WuXi Biology Global Facilities

USA



Boston, MA

Hit Finding (DEL, Peptide Discovery)
Compound Profiling (Biophysics)
Drug Delivery



San Diego, CA

Hit Finding
Compound Profiling
In Vitro Pharmacology
In Vivo Pharmacology
Bioanalysis

Germany



Munich

Protein Production
Structural Biology
Hit Finding (DEL, FBDD, SBDD)
Compound Profiling

China



Shanghai
(Waigaoqiao)

In Vitro Pharmacology, Immunology,
Oncology, Hepatology, Nephrology,
Infectious Disease, Biomarker Discovery



Shanghai
(Zhangjiang)

Target Discovery, Hit Finding,
Compound Profiling,



Nantong

Oncology, Neurobiology Cardiology,
Pulmonary Disease, Rare Disease,
Infectious Disease,
Reproductive and Endocrine Disease



Suzhou

Protein/Antibody Production,
Structural Biology, Oncology,
Compound Profiling,
Metabolic Disease

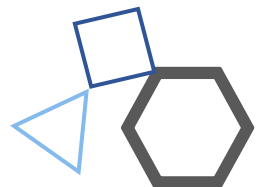


Chengdu

Drug Delivery, *In Vitro* Pharmacology,
Large Animal Pharmacology,
Radionuclide-Drug Conjugates Discovery,
Molecular Imaging Center

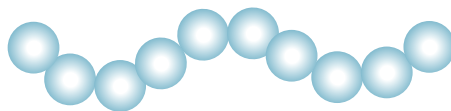
Locations for illustration only

Why Peptide as Potential New Modality Compound in Drug Discovery

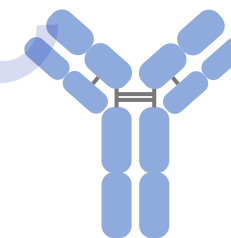


Small Molecules

<500 Da



Peptides



Proteins/Biologics

>5000 Da

Increasing Size & Complexity

Advantages Over Small Molecules

- High target specificity
- High target affinity
- Fewer side effects
- Less accumulation in tissues

General Advantages of Peptides

- Highly potent and selective
- Less toxic
- Low cost

Advantages Over Other Biologics

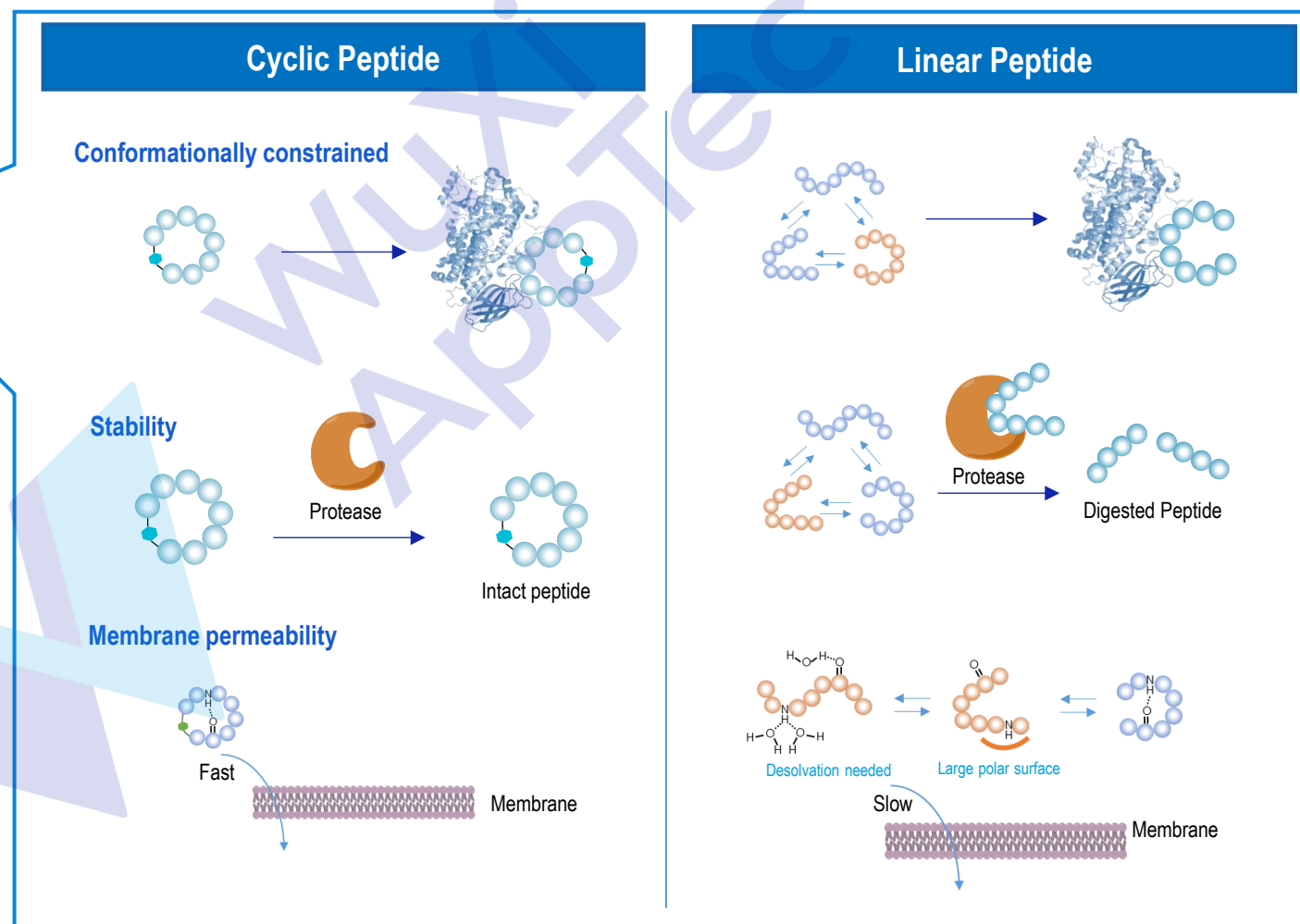
- High membrane permeability
- Less immunogenic
- Synthetically facile

Farooq, Z., Howell, L. A., & McCormick, P. J. (2022). Probing GPCR Dimerization Using Peptides. *Frontiers in endocrinology*, 13, 843770. <https://doi.org/10.3389/fendo.2022.843770>
Sharma, K., Sharma, K. K., Sharma, A., & Jain, R. (2023). Peptide-based drug discovery: Current status and recent advances. *Drug discovery today*, 28(2), 103464. <https://doi.org/10.1016/j.drudis.2022.103464>

Advantages of Cyclic Peptides

Advantages of Cyclic Peptides

- Better oral bioavailability
- Innate resistance to peptidase
- Allow optimization using standard small molecule chemical workflow

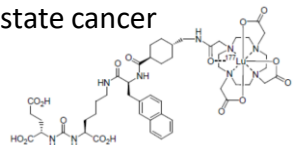


Peptide Therapeutic Area

Radionuclide drug conjugates

Pluvicto

- Radioligand therapeutic agent
- Advanced metastatic prostate cancer
- Natural ligand
- FDA approved

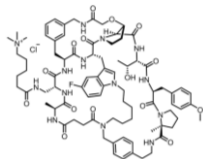


https://www.novartis.com/us-en/sites/novartis_us/files/pluvicto.pdf

Cardiovascular Disease

Enicitide chloride (MK-0616)

- PCSK9 inhibitor
- Hypercholesterolemia
- mRNA display
- Phase III

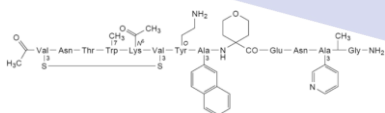


<https://drughunter.com/molecule/mk-0616>

Immune System

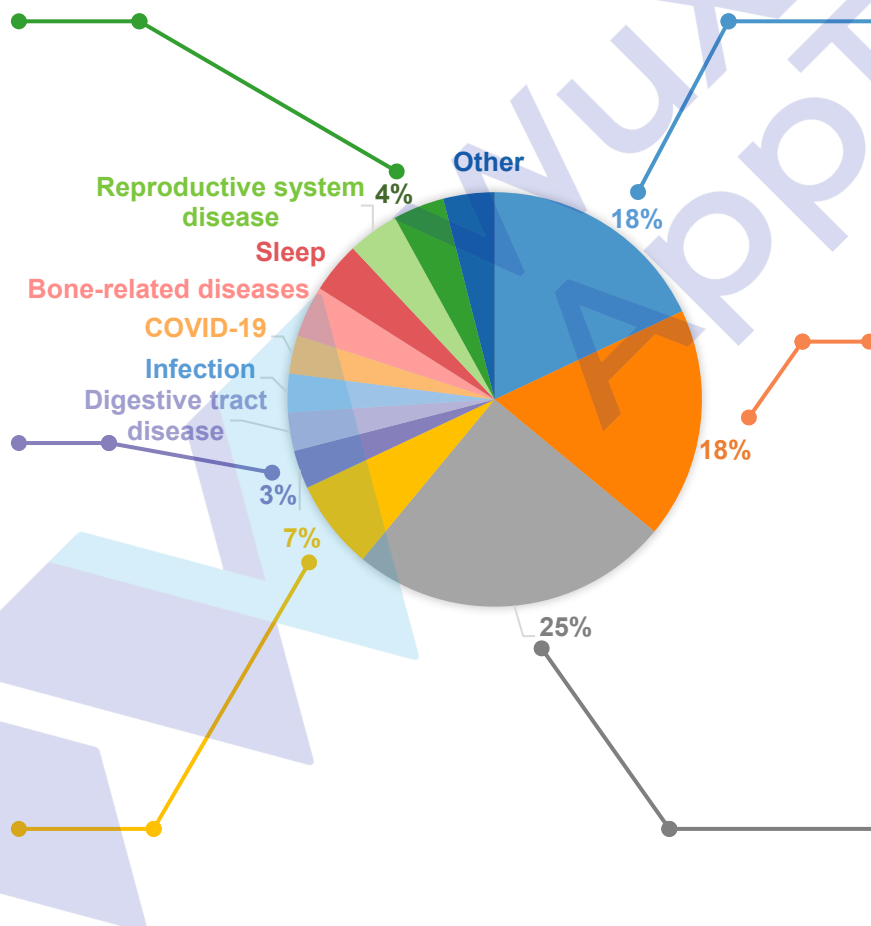
Icotrokinra (JNJ-77242113)

- IL-23 receptor antagonist
- Moderate to severe plaque psoriasis
- Phage display
- Phase III



<https://doi.org/10.1038/s41598-024-67371-5>

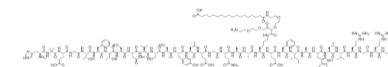
Distribution of peptide-based drugs indication from 2014 to 2024



Diabetes mellitus and its complications

Semaglutide

- GLP-1 receptor agonist
- Type 2 diabetes and obesity
- Natural ligand
- FDA approved

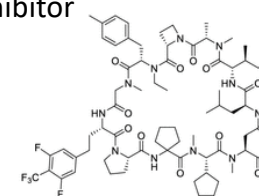


<https://www.medkoo.com/products/57427>

Cancer

Palurptide (LUNA18)

- cyclic peptide pan-RAS inhibitor
- Solid tumors
- mRNA display
- Phase I

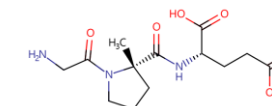


<https://doi.org/10.1021/jacs.3c03886>

Rare diseases

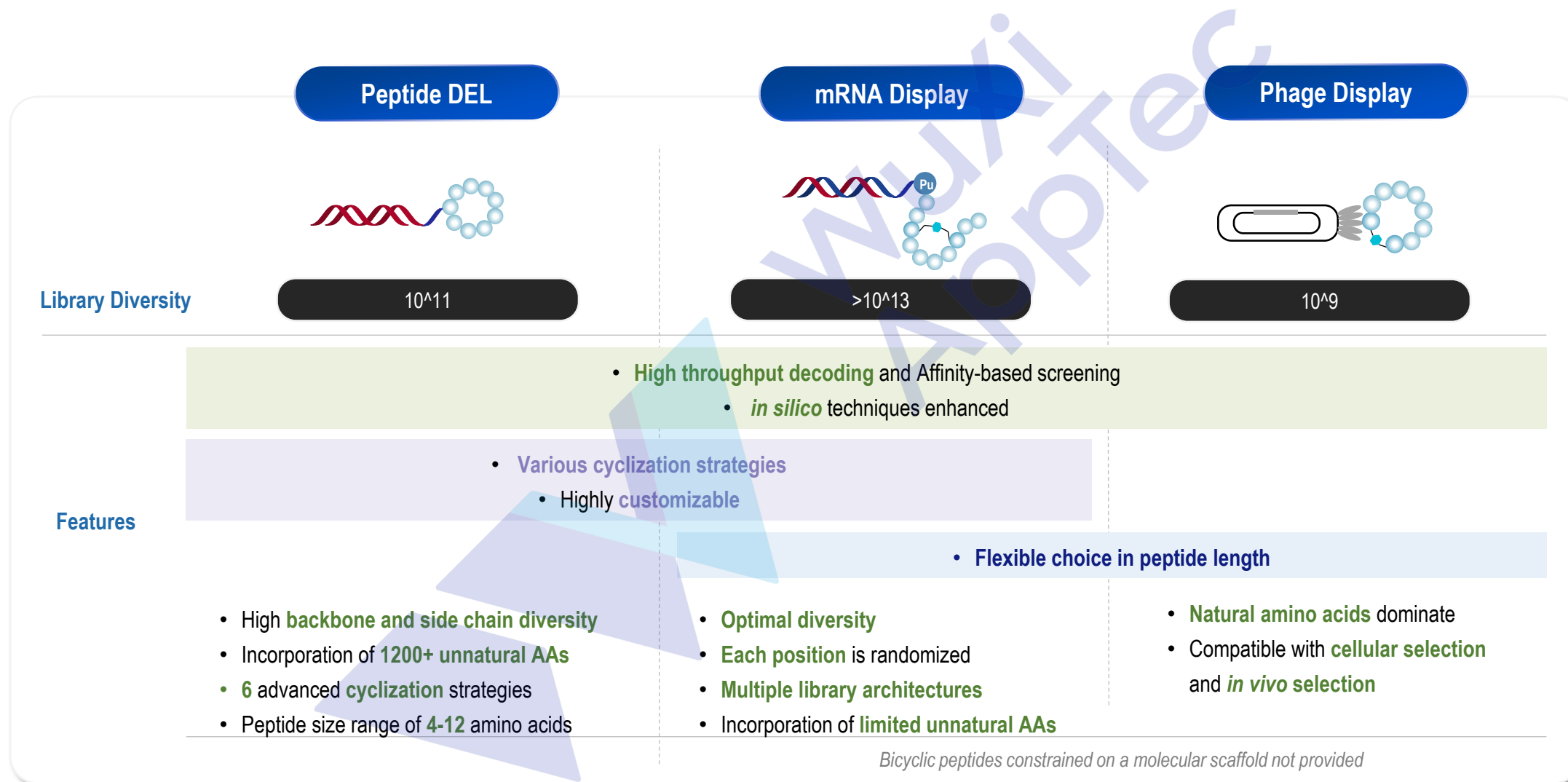
Daybue (trofinetide)

- IGF-1R agonist
- Rett syndrome (a rare, genetic neurological disorder)
- Natural ligand
- FDA approved



<https://go.drugbank.com/drugs/DB06045>

DEL and Display for de novo Peptide Discovery

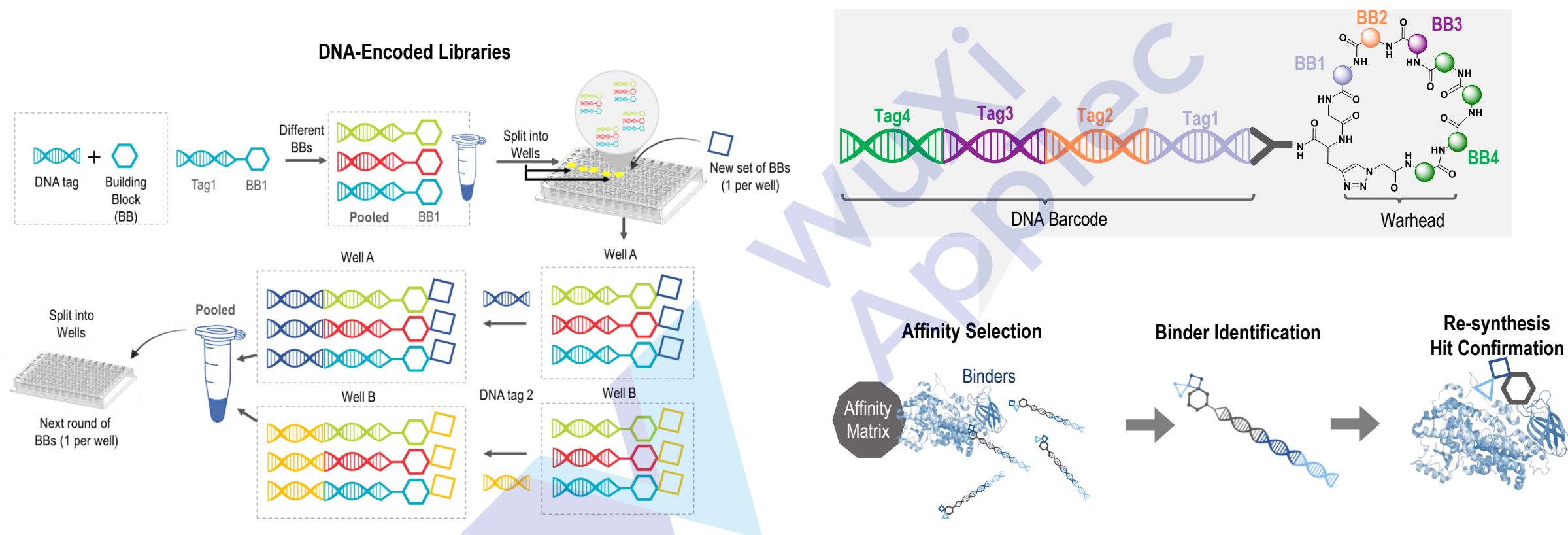


01



Part 1: Peptide DNA-Encoded Library (DEL)

A Typical DEL Approach



- Combinatory Synthesis and Bar-coded by DNA
- Extremely Large Library Size with Extremely Low Cost-per-compound
- Sequence-based De-convolution Enables One-pot Screening

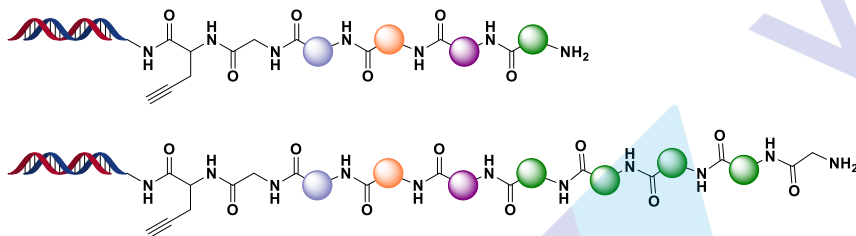
- Minimum Assay Development
- Fast Turn-over and Low Reagent Consumption
- Multiple Channels in Parallel in One Campaign
- Rich SAR Information

Peptide DEL Library Formula – Super Pool 1.0

Total diversity: ~260B

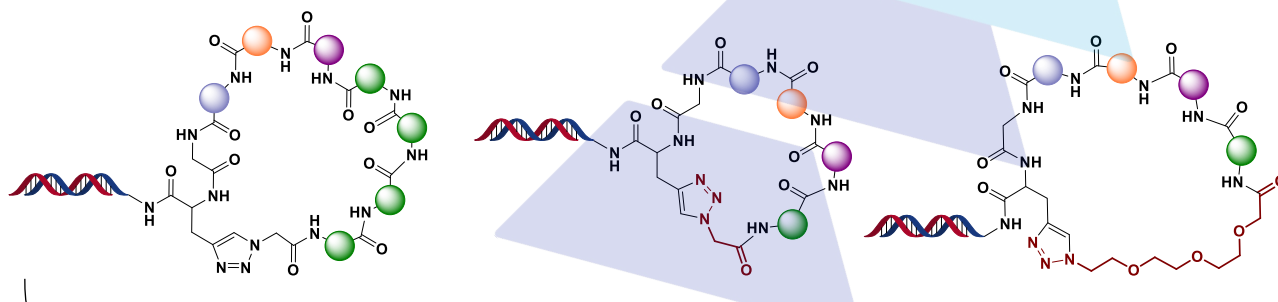
Chemical Structures of Peptide DEL

Linear Peptide Libraries



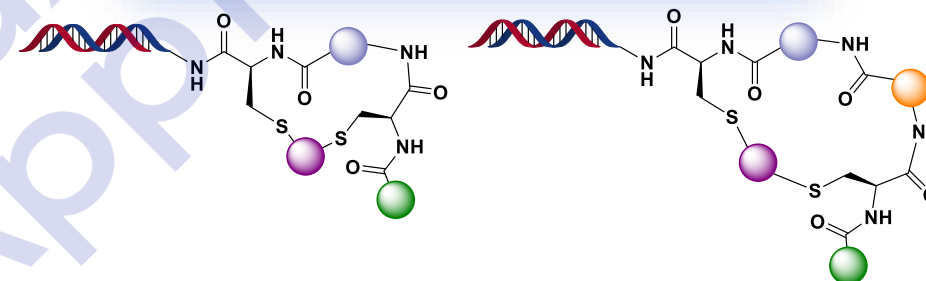
Total diversity: ~95 Billion

Cyclic Peptide Libraries



Total diversity: ~156 Billion

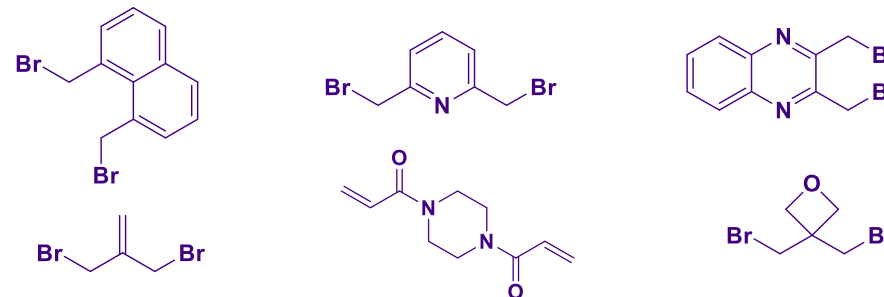
Small Thioether Cyclized Peptide DEL



Diversity: ~60 Million

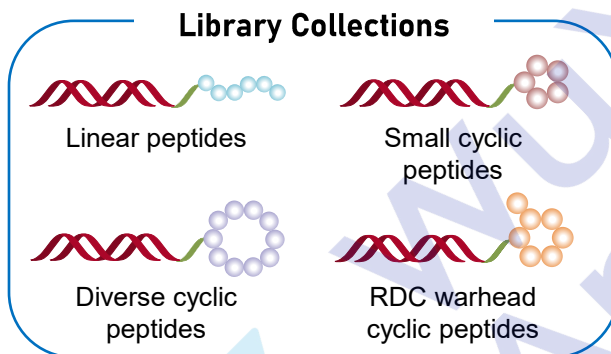
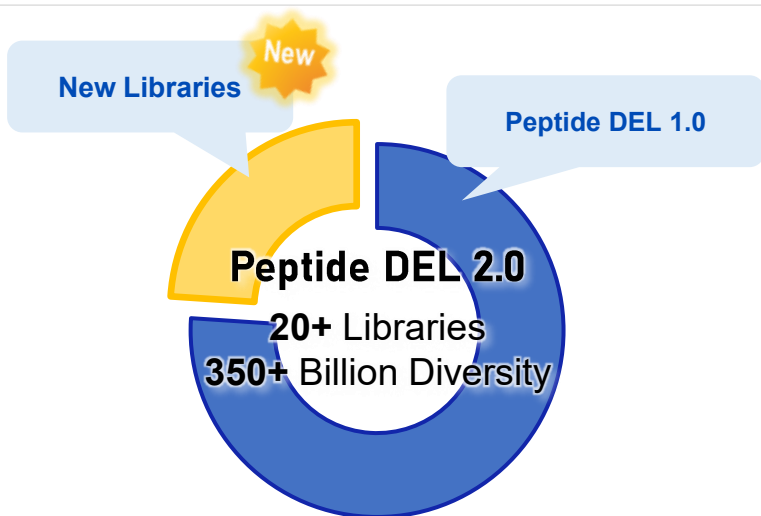
Diversity: ~51 Billion

Representative Linkers



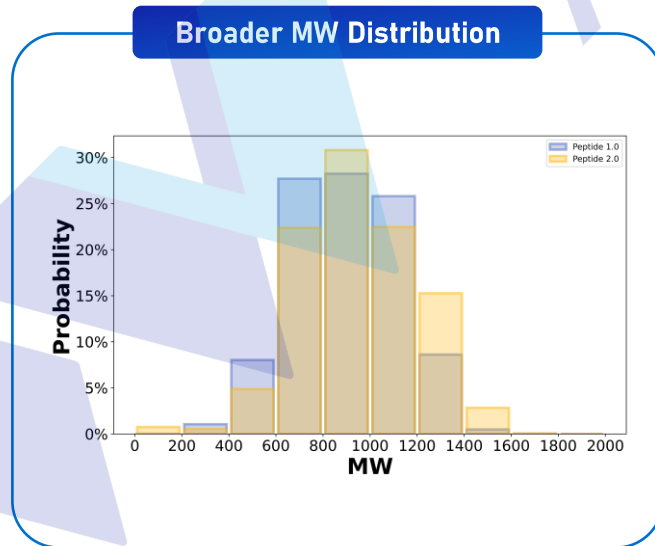
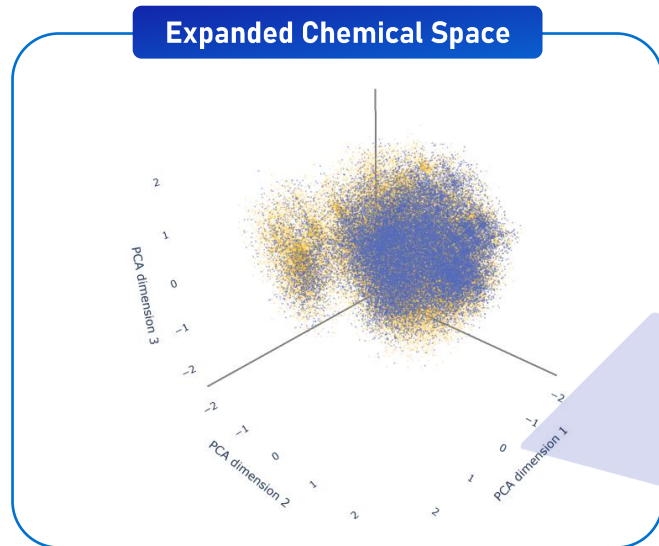
Total number of linkers: 22

Launch of Peptide DEL 2.0



Upgraded Libraries

- Broader peptide size range: **4AAs to 12AAs**
- Expanded chemical space
- Natural AAs and **1200+ unnatural AAs**, including D-/L-isomers, α N-alkylation and α - β - γ - AAs
- **6** cyclization strategies



● Peptide DEL 1.0 ● Peptide DEL 2.0

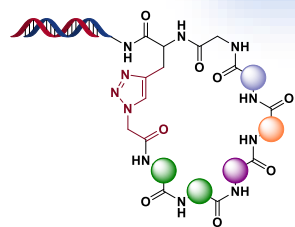
In-depth Experience

- >50 targets screened
- >10 target classes across various therapeutic areas
- >70% screening success rate

Peptide DEL Library – Super Pool 2.0

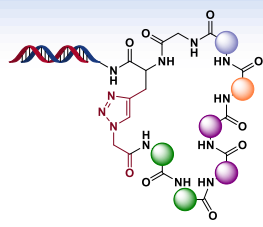
Total diversity: ~86B

Cyclic Peptide libraries



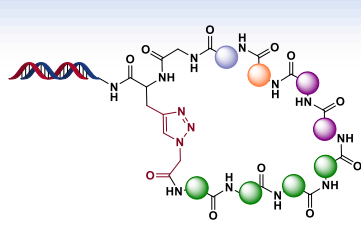
Diversity: ~11 Billion

Cyclization:
CuAAC click
reaction



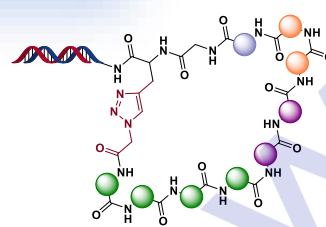
Diversity: ~2.1 Billion

Cyclization:
CuAAC click
reaction



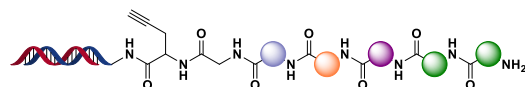
Diversity: ~2.3 Billion

Cyclization:
CuAAC click
reaction

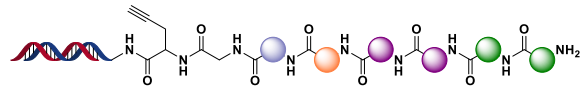


Diversity: ~0.42 Billion

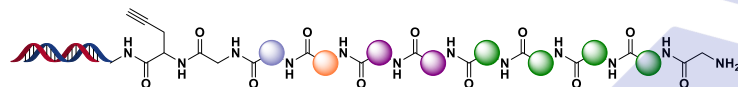
Cyclization:
CuAAC click
reaction



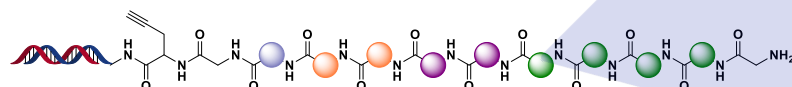
Diversity: ~11 Billion



Diversity: ~2.1 Billion

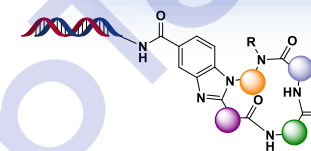


Diversity: ~2.3 Billion

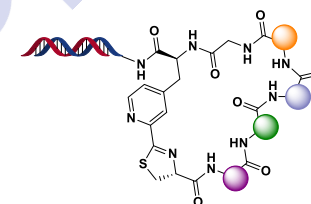


Diversity: ~0.42 Billion

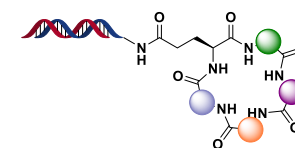
Advanced Cyclization Strategy & Small Cyclic Peptide Libraries



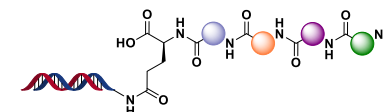
Diversity: ~12.7 Billion
Cyclization: Benzimidazole



Diversity: ~1.6 Billion
Cyclization: Nitrile-aminothiol
Click reaction



Diversity: ~20 Billion
Cyclization: Amidation



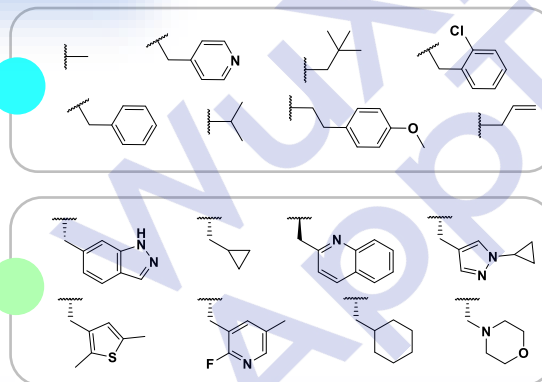
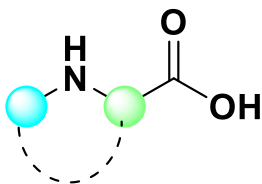
Diversity: ~20 Billion
Cyclization: /

Amino Acid Selection for Custom Peptide DEL

Key Features of Peptide DEL about AA

- ✓ **1,400+** validated natural and unnatural amino acids
- ✓ **1000+** α -amino acids with diverse side chains
- ✓ **300+** non α -amino acids with diverse backbones
- ✓ **10%** amino acids with α N-alkylation

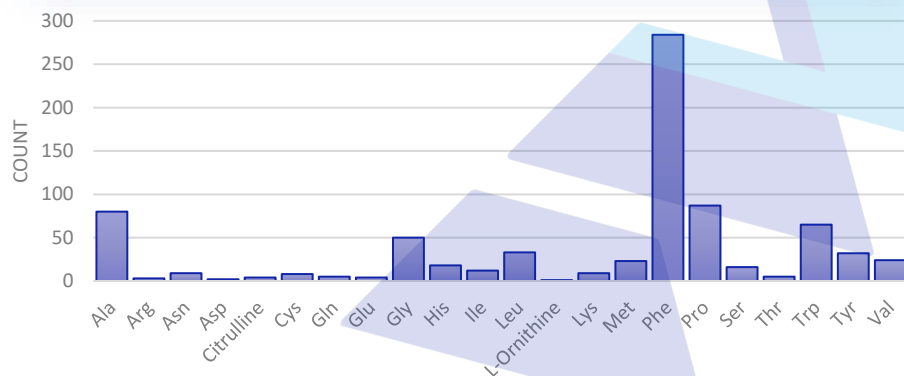
Amino Acid Collection



Selection Strategies

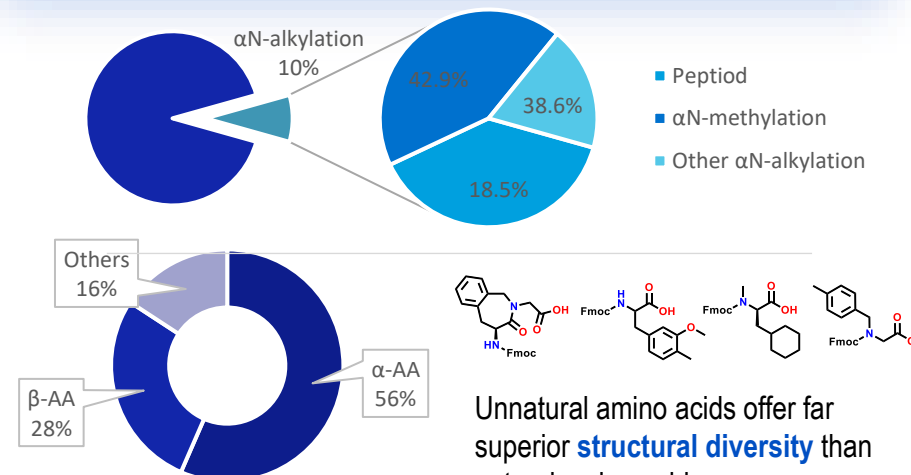
- ✓ α N-alkylation AA: Enhanced Membrane Permeability & Improved DMPK
- ✓ β -AA: Increased Proteolytic Resistance & Enhanced Metabolic Stability
- ✓ Charged AA: Membrane Permeability Modulation & Solubility Modulation
- ✓ Site-specific AA substitutions to modulate H-bond donors/acceptors

Natural Amino Acids and Derivatives



Abundant natural amino acids and their derivatives enhance the **structural optimization** and **modification** of natural peptides.

Unnatural Amino Acids



Unnatural amino acids offer far superior **structural diversity** than natural amino acids.

Case Study: MDM2-p53 Inhibitor Discovery Using Peptide DEL

ACS Medicinal Chemistry Letters

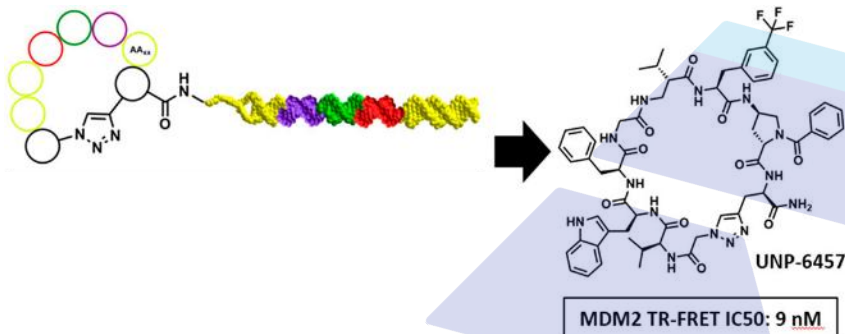
pubs.acs.org/acsmchemlett

Letter

DNA-Encoded Macrocyclic Peptide Libraries Enable the Discovery of a Neutral MDM2-p53 Inhibitor

4.5-billion-member cyclic peptide DEL library:

- Highly non-proteinogenic
- High backbone diversity
- Lower MW, TPSA, HBD and HBA count than phage and mRNA display



Joshua Schwochert • 2 度
Co-founder and CSO of Unnatural Products
9 个月前 •

+ 关注 ...

<https://lnkd.in/gcy7Y3EW>

Congrats **Anthony Silvestri** and team. Excited to announce our publication in collaboration with WuXi, in particular **Wenji Su**, on cyclic peptide DNA-encoded libraries. We think DEL offers some unique advantages over ribosomally encoded libraries, which have traditionally dominated the peptide discovery space. Looking forward to seeing where this technology can take us when applied to novel, unaddressed targets.

Here's to continued innovation in the peptide discovery space as the field builds momentum.

[查看译文](#)

Joshua Schwochert

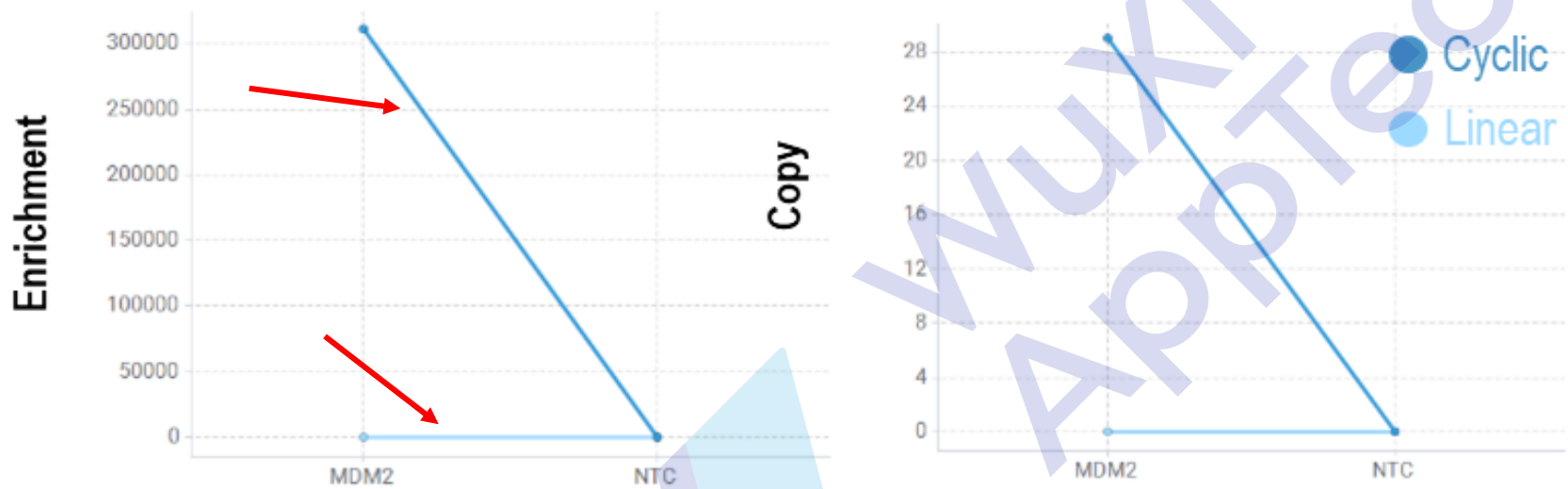
Co-founder and CSO of Unnatural Products

 Unnatural Products

We think DEL offers some unique advantages over ribosomally encoded libraries, which have traditionally dominated the peptide discovery space. Looking forward to seeing where this technology can take us when applied to novel, unaddressed targets.

Silvestri, A. P., Zhang, Q., Ping, Y., Muir, E. W., Zhao, J., Chakka, S. K., Wang, G., Bray, W. M., Chen, W., Friborough, J. L., Tripathi, S., He, Y., Rubin, S. M., Satz, A. L., Pye, C. R., Kuai, L., Su, W., & Schwochert, J. A. (2023). DNA-Encoded Macrocyclic Peptide Libraries Enable the Discovery of a Neutral MDM2-p53 Inhibitor. *ACS medicinal chemistry letters*, 14(6), 820–826. <https://doi.org/10.1021/acsmchemlett.3c00117>

Case Study: Direct Comparison of Cyclic & Linear Peptide using Sub-libraries in Peptide-DEL



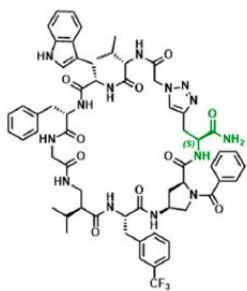
NsynthonID	Copy (MDM2)	Copy (NTC)	Enrichment (MDM2)	Enrichment (NTC)
10577-401-415-375-180	0	0	0.1	0.1
10578-401-415-375-180	0	0	0.1	0.1
10579-401-415-375-180	29	0	311417.56	0.1

Silvestri, A. P., Zhang, Q., Ping, Y., Muir, E. W., Zhao, J., Chakka, S. K., Wang, G., Bray, W. M., Chen, W., Fribourgh, J. L., Tripathi, S., He, Y., Rubin, S. M., Satz, A. L., Pye, C. R., Kuai, L., Su, W., & Schwachert, J. A. (2023). DNA-Encoded Macrocyclic Peptide Libraries Enable the Discovery of a Neutral MDM2-p53 Inhibitor. *ACS medicinal chemistry letters*, 14(6), 820–826. <https://doi.org/10.1021/acsmedchemlett.3c00117>

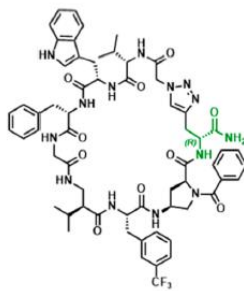
Case Study: Validation of Hits Identified from Peptide DEL Screening

Bioassay Results

- UNP-6456 and UNP-6457 were synthesized and tested in relevant assays

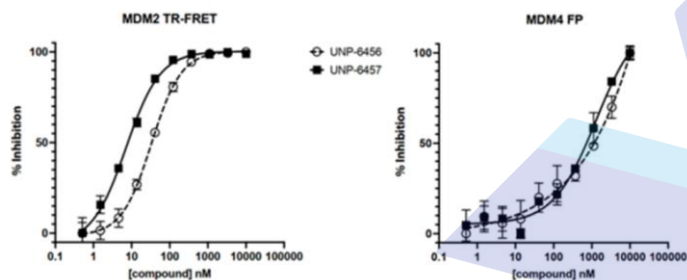


UNP-6456



UNP-6457

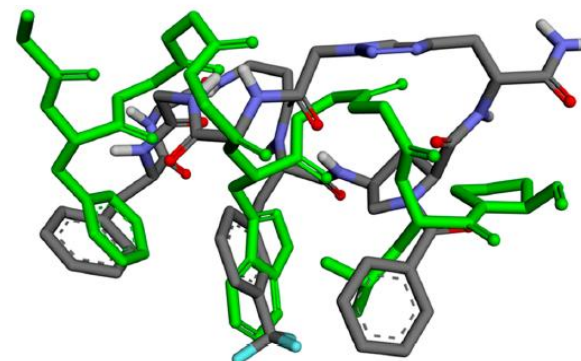
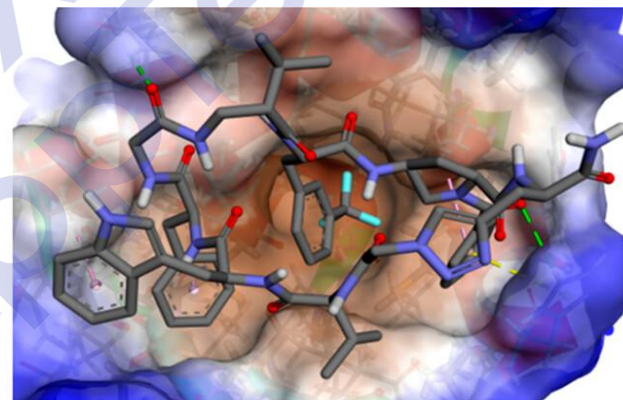
- Synthesized hits shows strong potency as MDM2-p53 protein-protein interaction inhibitor



n=2	UNP-6456	UNP-6457
hMDM2 TR-FRET IC50 (μM)	0.043	0.009
hMDM4 FP IC50 (μM)	5.15	3.30
PAMPA (x10 ⁻⁶ cm/s)	< 0.00	< 0.00
RS4;11 viability IC50 (μM)	> 50.0	> 50.0

X-ray Structural Analysis

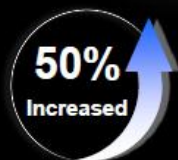
- X-ray structural analysis of the MDM2-Compound complex



Academic Access to Peptide DEL



Coming Soon: The Next-Generation DELopen



6.3 Billion

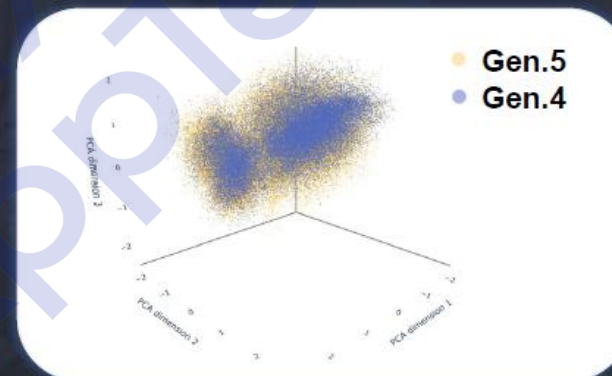
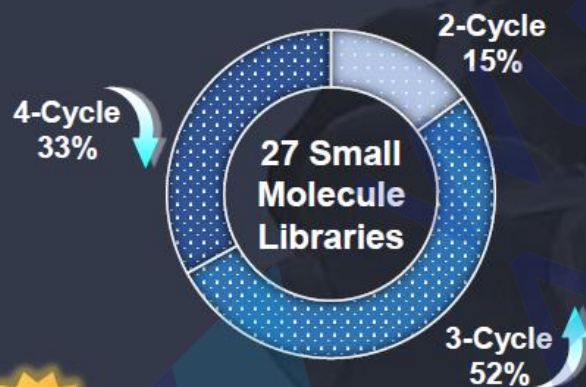
Small Molecule Library



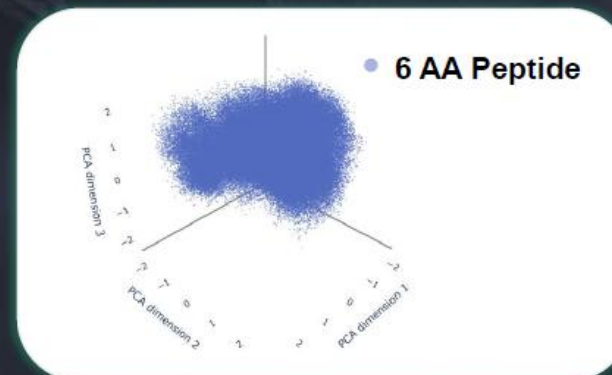
0.27 Billion

Peptide DEL Library

Small Molecule Library - Advanced Chemical Diversity



Extra Peptide DEL - More Options for Your Research



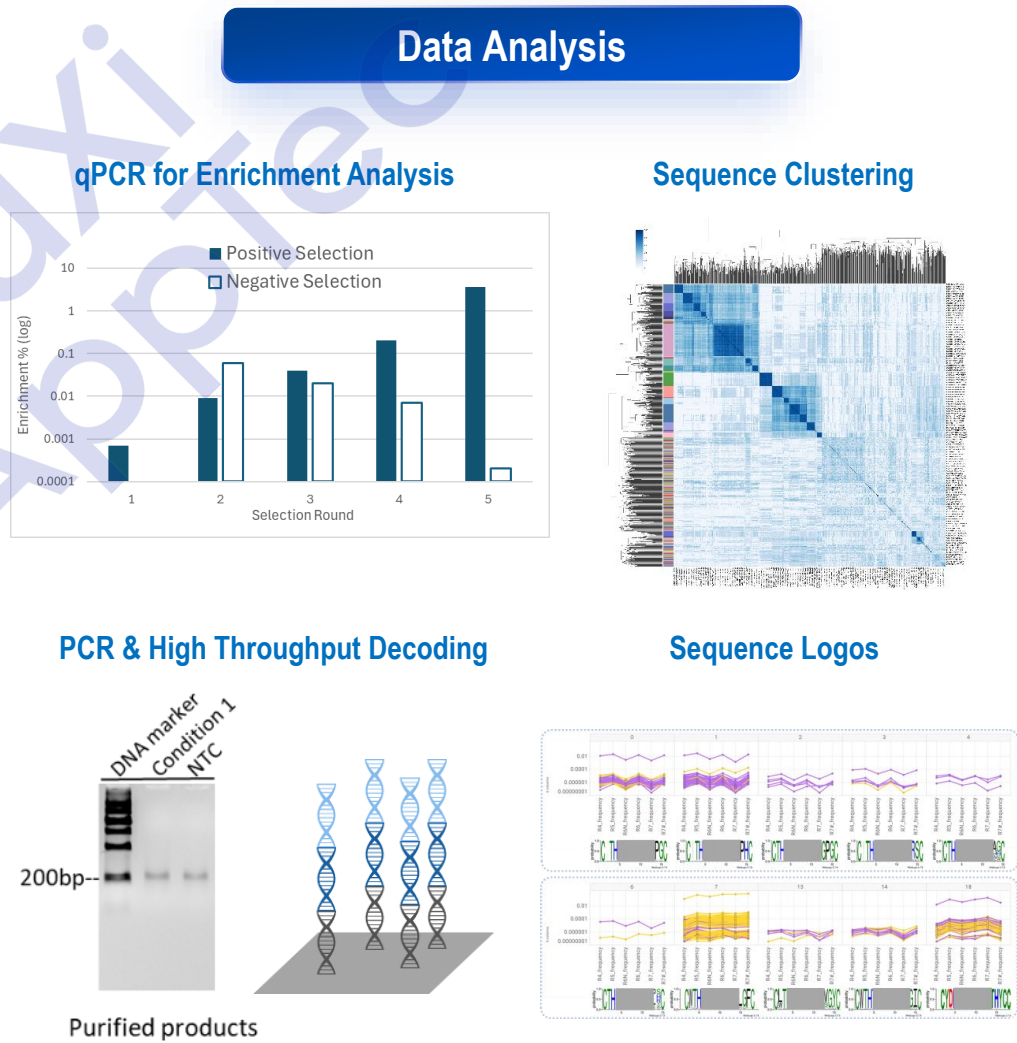
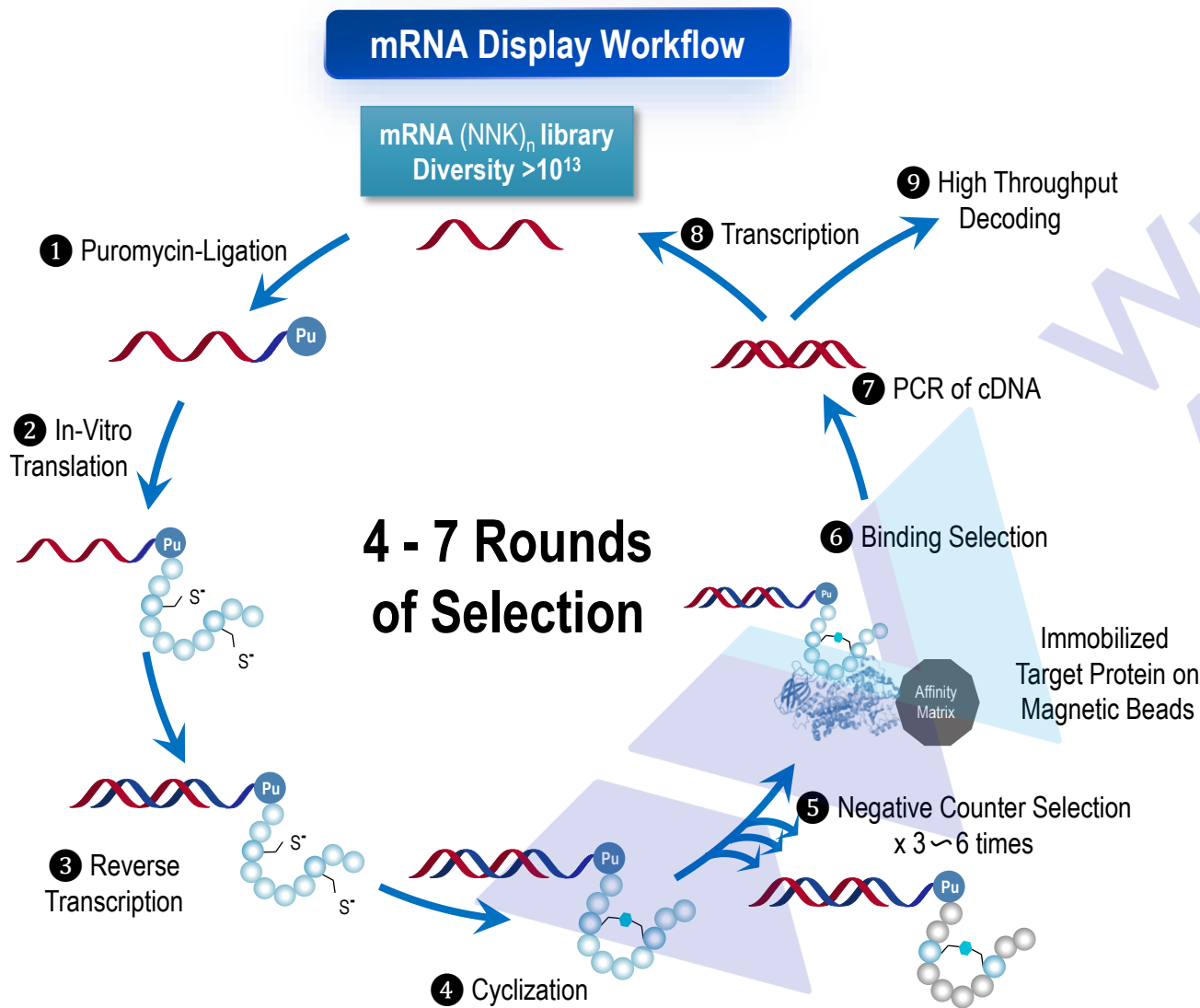
Pre-register for the 5th Gen DELopen Kit via email: DELopen_Platform@wuxiapptec.com

02



Part 2: mRNA Display

mRNA Display Workflow

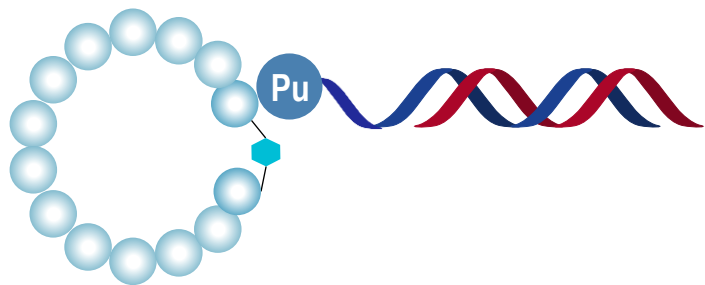


mRNA Display Peptide Libraries

Cyclic peptide library, natural AA only

Off-the-shelf Library

➤ Peptide Structure



➤ Library Construct

AUG UGC (NNK/XXX)_n UGC
Met Cys (XXX)_n Cys
Where n = 6-15

Note:

- 1. N = G, A, C T, K = T, G
- 2. XXX: Pre-synthesized Trimer Phosphoramidites without Cys

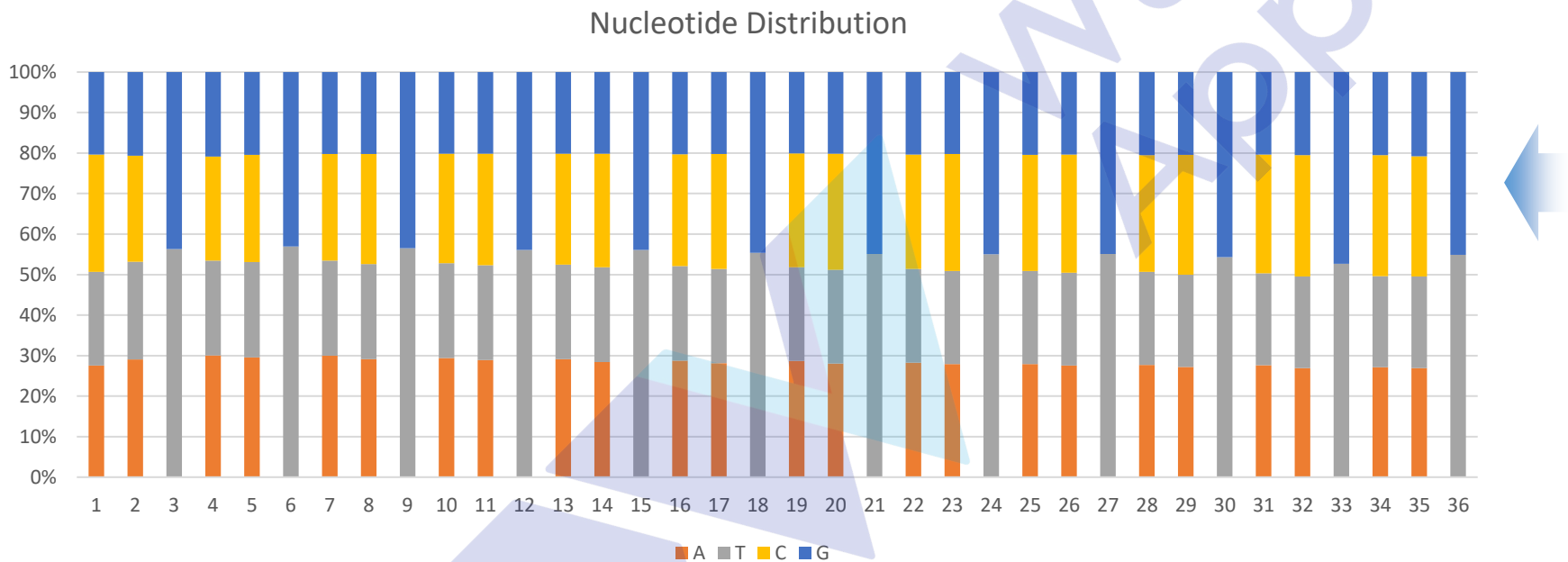
Two Strategies for Oligonucleotide Synthesis

➤ Codons for customized library

Library Construct	NNK	Trimer
Features	Degenerate codons	Pre-synthesized trinucleotides
	32 codons for 20 amino acids	20 trinucleotides coding 20 amino acid
	Uneven distribution of amino acid	Controlled ratio for amino acid

mRNA Library High Throughput Decoding QC

QC result for 12mer library



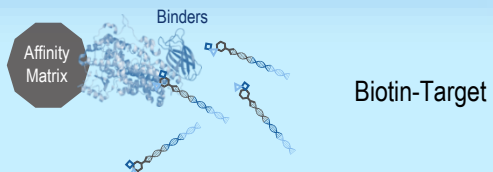
postion	A	T	C	G
1	0.275716	0.230888	0.290127	0.203269
2	0.290886	0.241168	0.261631	0.206314
3	0	0.562927	0	0.437073
4	0.300797	0.233966	0.256922	0.208316
5	0.295355	0.235389	0.265011	0.204244
6	0	0.569384	0	0.430616
7	0.299626	0.234735	0.263792	0.201847
8	0.291031	0.234927	0.271793	0.202249
9	0	0.565212	0	0.434788
10	0.294374	0.233923	0.27026	0.201443
11	0.288768	0.234198	0.275239	0.201795
12	0	0.561198	0	0.438802
13	0.291512	0.233013	0.274318	0.201157
14	0.284331	0.233632	0.280607	0.201431
15	0	0.561007	0	0.438993
16	0.28772	0.23365	0.2759	0.202731
17	0.281501	0.232361	0.283899	0.202238
18	0	0.553574	0	0.446426
19	0.286887	0.231336	0.281154	0.200622
20	0.280673	0.2313	0.286684	0.201343
21	0	0.55083	0	0.44917
22	0.282833	0.231328	0.282168	0.203672
23	0.278948	0.229994	0.288647	0.202411
24	0	0.550208	0	0.449792
25	0.278852	0.229794	0.286793	0.204561
26	0.275503	0.229409	0.291553	0.203534
27	0	0.550799	0	0.449201
28	0.277157	0.229548	0.288019	0.205276
29	0.272216	0.227583	0.295542	0.204659
30	0	0.542849	0	0.457151
31	0.276367	0.22718	0.292556	0.203898
32	0.269355	0.225851	0.299603	0.205191
33	0	0.526682	0	0.473318
34	0.271361	0.225097	0.29883	0.204712
35	0.269095	0.226461	0.296594	0.20785
36	0	0.54886	0	0.45114

- A/T/C/G frequency ranges from 20% to 30% in position N.
- G/T frequency ranges from 41% to 59% in position K.

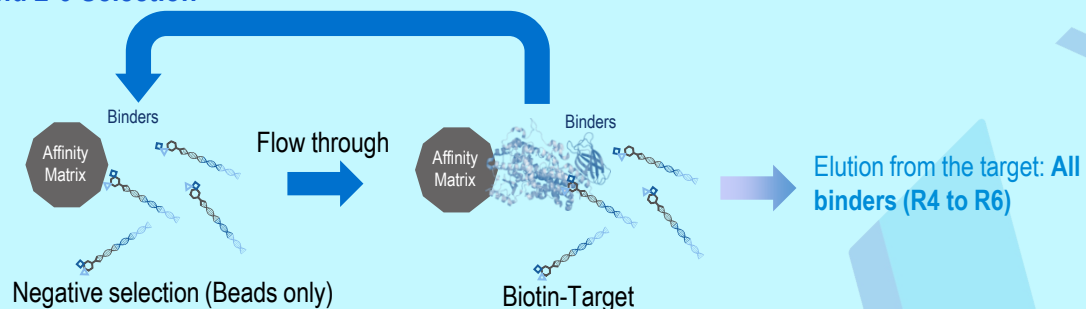
Case Study - mRNA Display Screening Performance

Selection Strategy

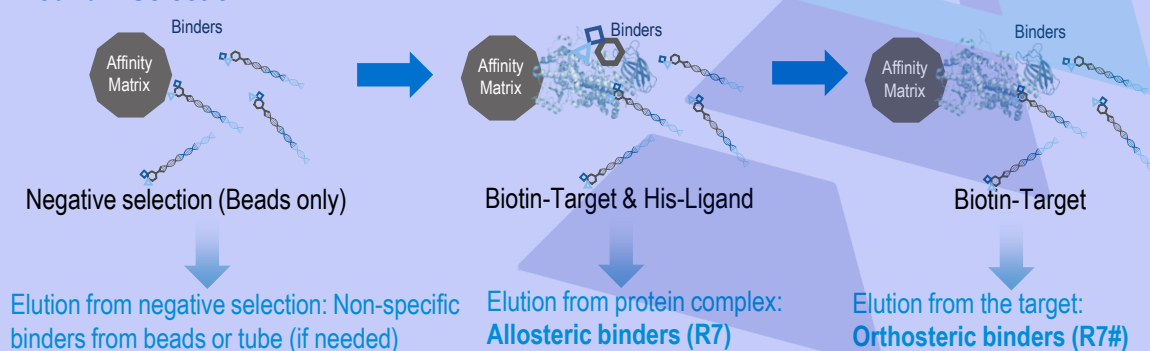
Round 1 Selection



Round 2-6 Selection

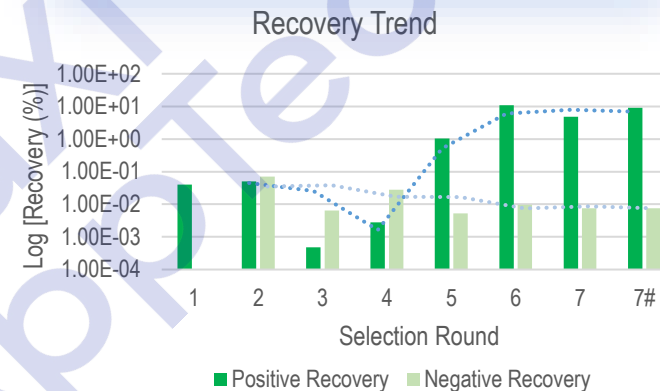


Round 7 Selection



- Negative selection: 3 times, to remove non-specific binders

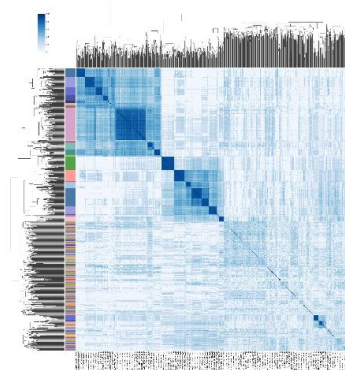
Selection Performance



- The target shows a good enrichment trend

Identity Matrix with Score $\geq 40\%$

Heatmap



Line plot & Sequence Logo

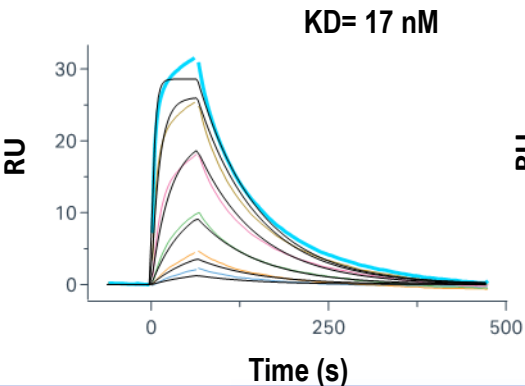


- 19 sequence clusters are observed with count number ≥ 10
- 21 peptides from clusters with good performance were picked for validation

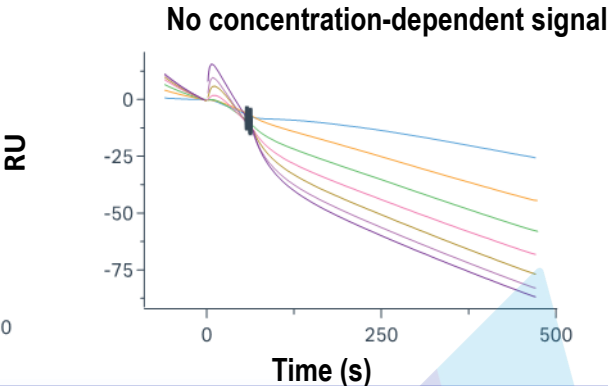
mRNA Display Validation Results & Oligo Delivery Efficiency

Peptide 1: Orthosteric Binder

Peptide 1: Target (1:1 binding kinetic)

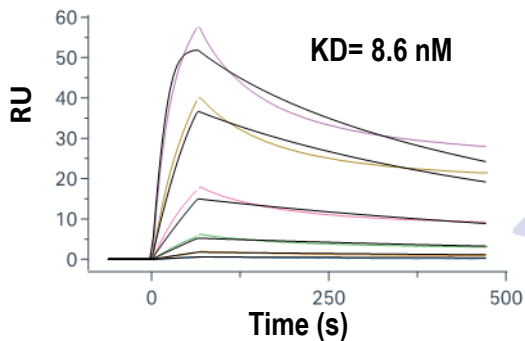


Peptide 1: Target/Ligand (1:1 binding kinetic)

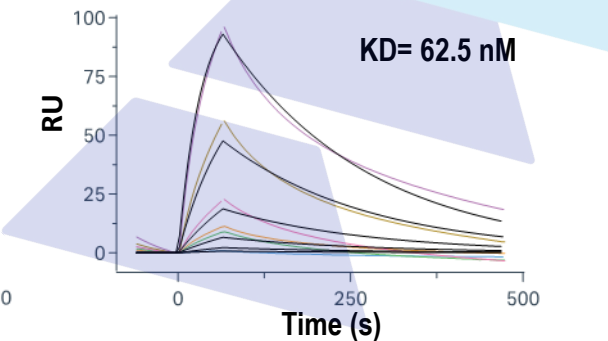


Peptide 2: Allosteric Binder

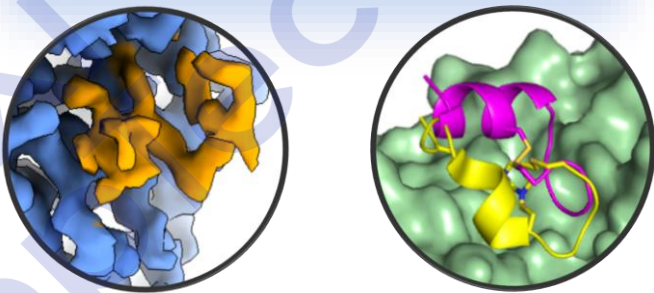
Peptide 2: Target (1:1 binding kinetic)



Peptide 2: Target/Ligand (1:1 binding kinetic)

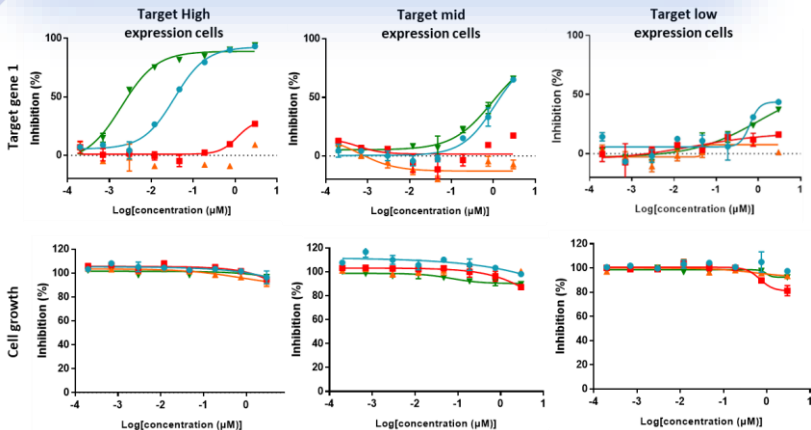


Structural Analysis



➤ Cryo-EM confirmed allosteric binding

Knockdown Efficiency Evaluation

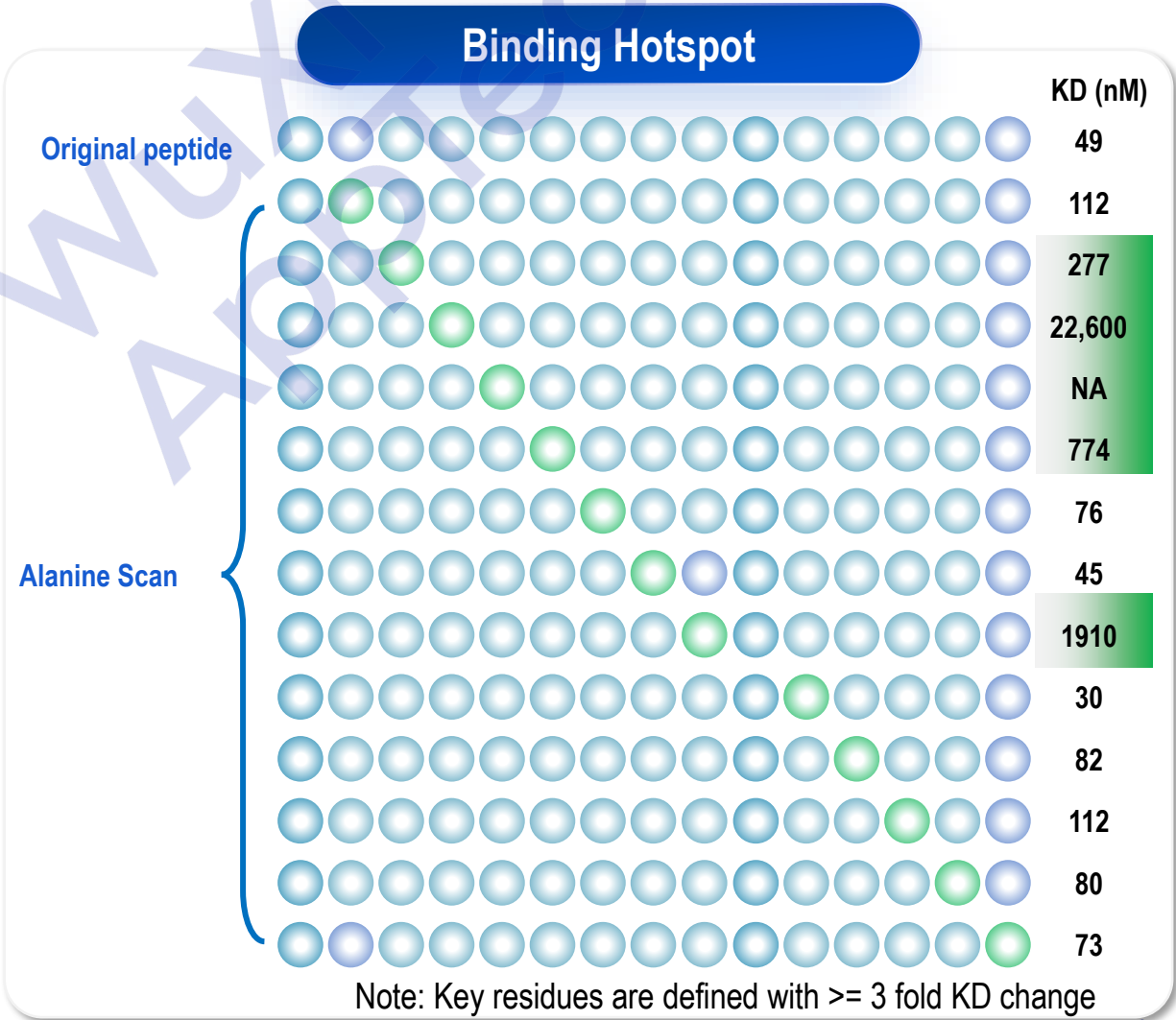
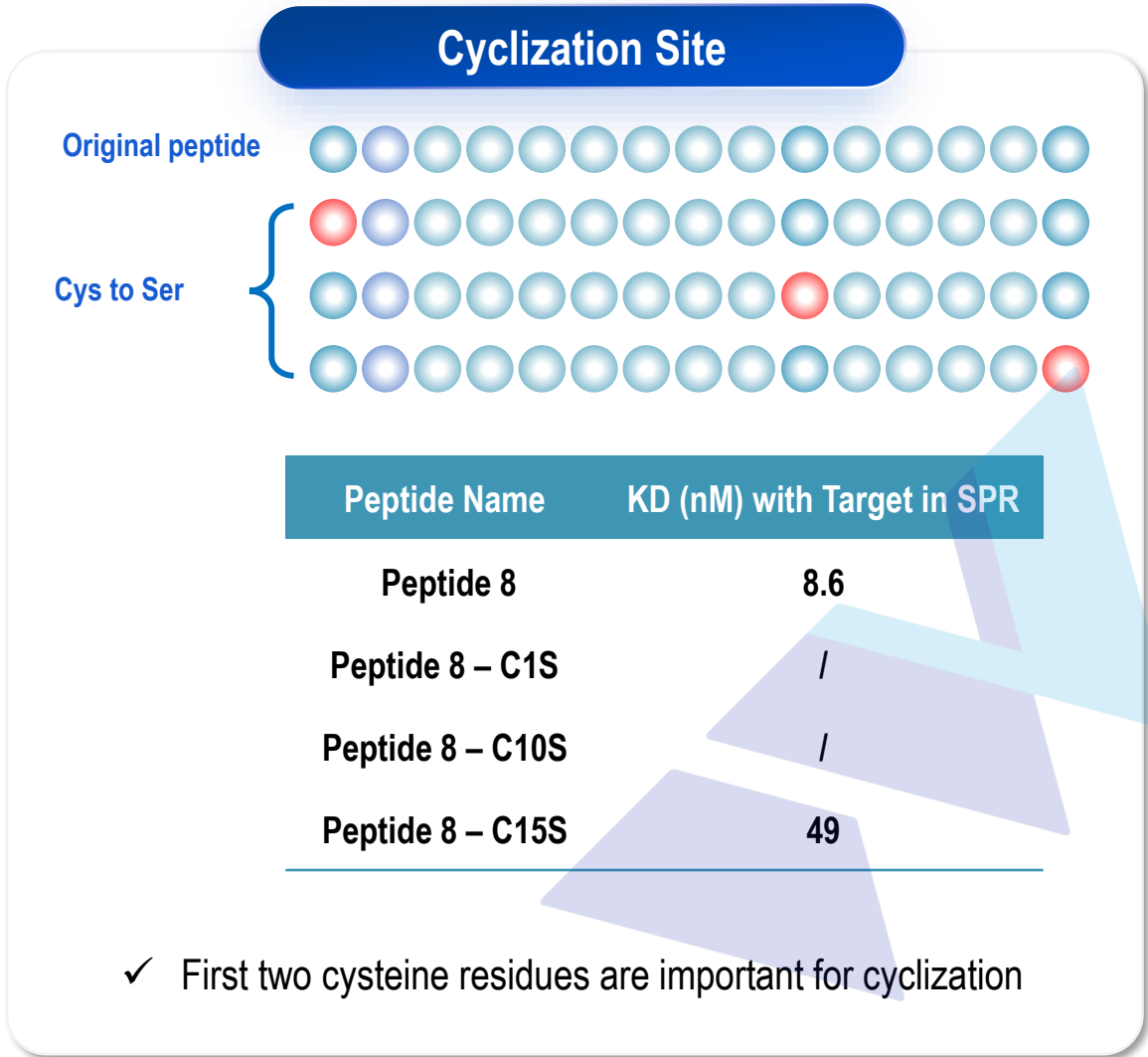


- WuXi peptide-SiHPRT1
- SiHPRT1 only
- ▲ WuXi peptide only
- ▼ Benchmark SiHPRT1

IC ₅₀	
WuXi	37nM
Benchmark	2.4nM

Case Study - mRNA Display Validation Results

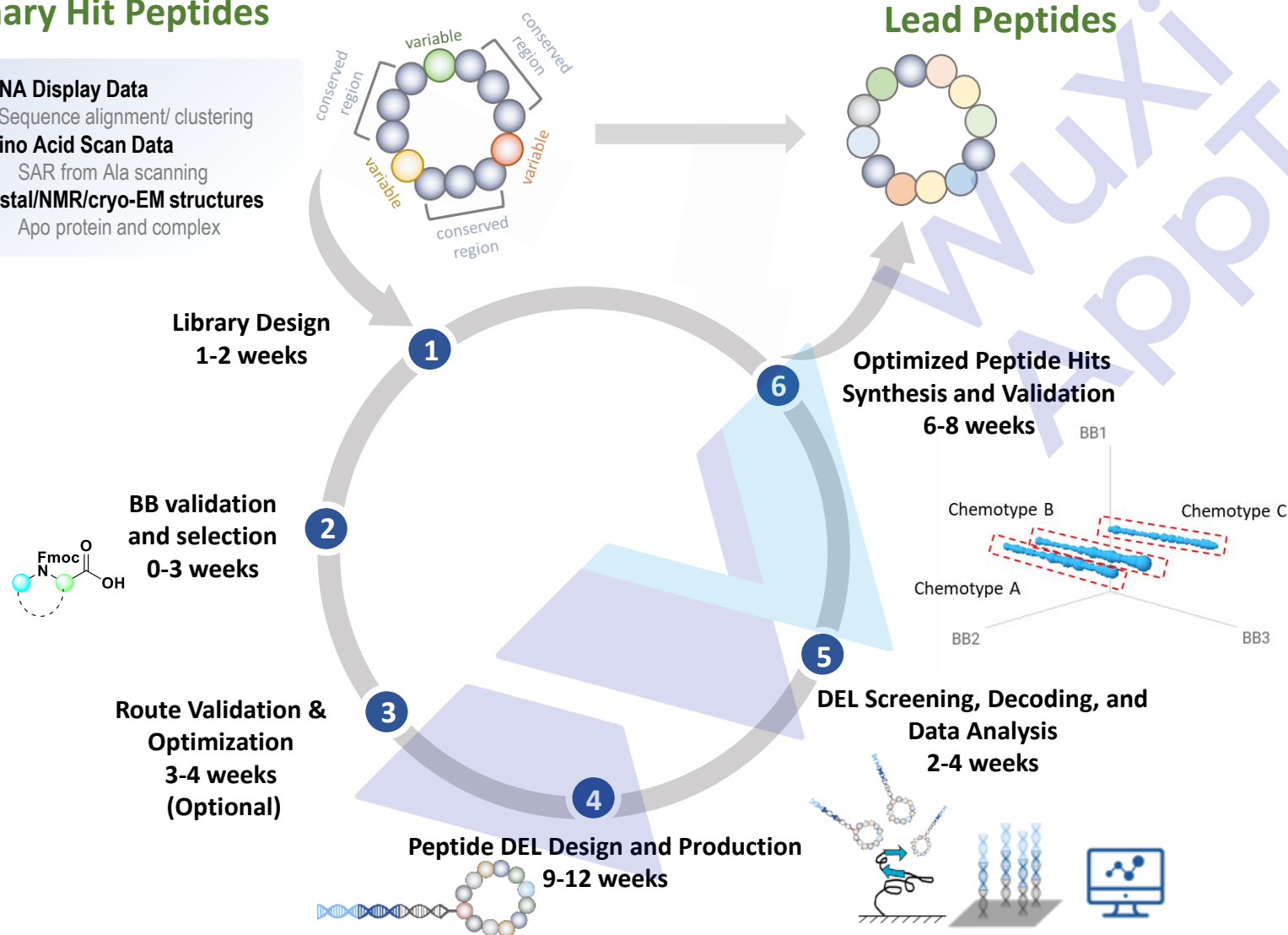
Confirmation for Cyclization Site and Binding Hotspot using SPR



Workflow of Customized Focus Peptide DEL for Hit-to-Lead Optimization

Primary Hit Peptides

- ★ mRNA Display Data
 - Sequence alignment/ clustering
- ★ Amino Acid Scan Data
 - SAR from Ala scanning
- ★ Crystal/NMR/cryo-EM structures
 - Apo protein and complex



Showcase

Timeline: **7 Months**

Library Diversity:

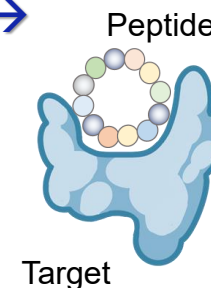
700*700*700 UAAs

>300 Fold

Improvement in Affinity from
3 separate chemotypes

From single digit nM →

pmol
binding affinity



03



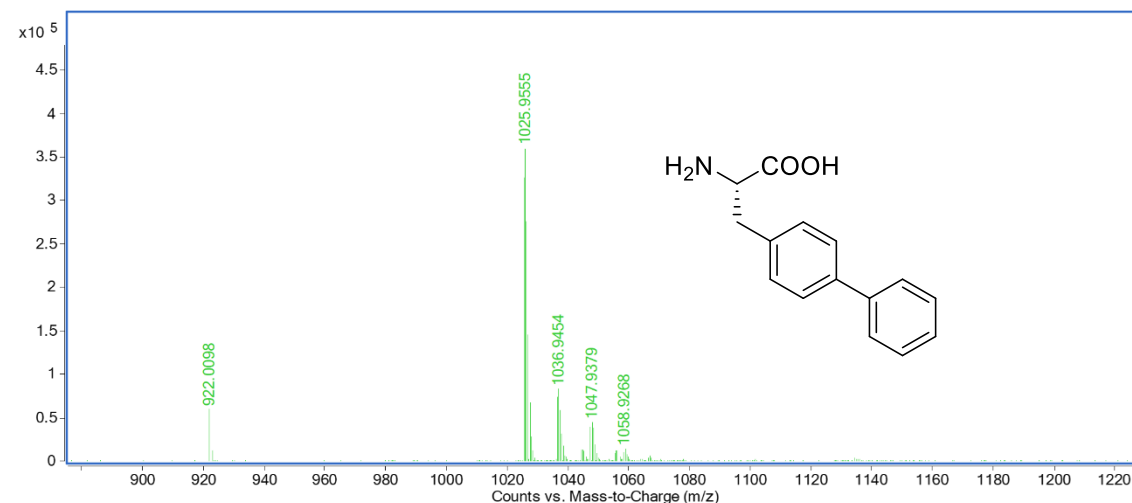
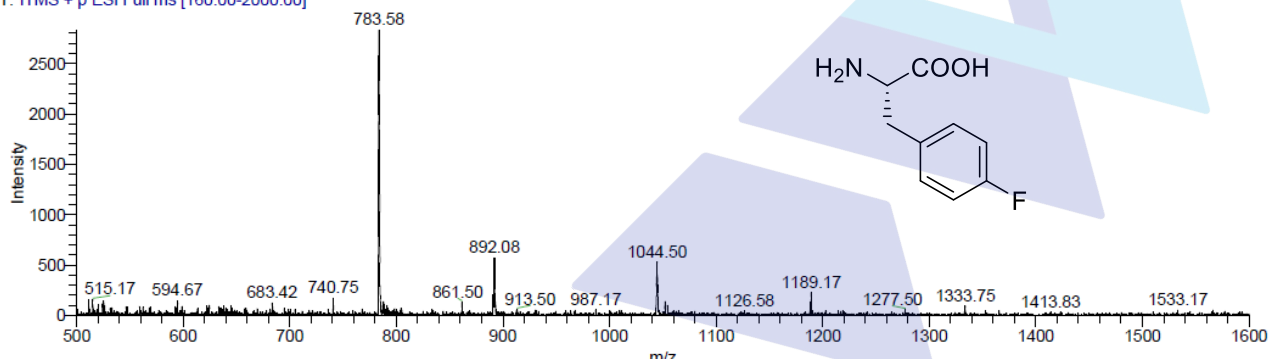
Part 3: Extended Capabilities

Genetic Code Expansion via Stop Codon Suppression

Incorporation of unnatural amino acids via UAG stop codon

- Validated two approaches for incorporation into model peptides with no detectable misincorporation
- POC incorporation of **4-fluoro-L-phenylalanine** using aminoacyl-tRNA synthetase charging of tRNA
- POC incorporation of **biphenylalanine** using pdCpA ligation of tRNA
- Ongoing efforts to expand the range of non-natural amino acids accessible using WuXi AppTec's in-house IVT system**

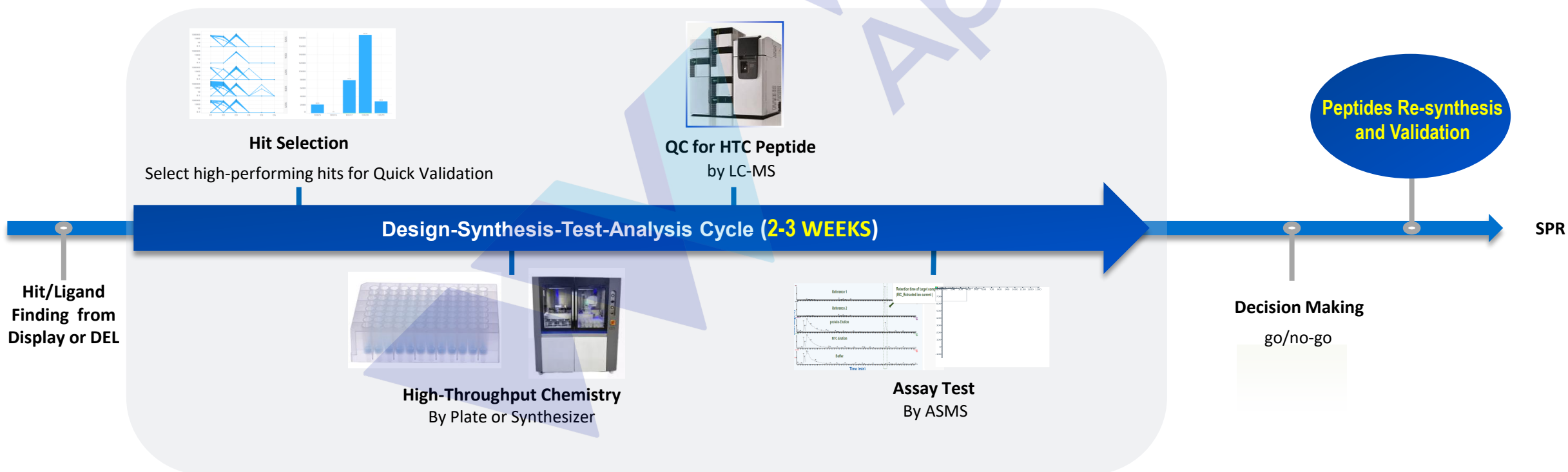
MP1_HABead #170-173 RT: 1.13-1.15 AV: 4 NL: 2.83E3
T: ITMS + p ESI Full ms [160.00-2000.00]



What is the HTC Peptide Platform, and why choose it?

High-Throughput Peptide Synthesis Platform for Hit Validation: Per validated throughput: UAAs > 48 → HTC on-plate synthesis; UAAs < 48 → SPPS on synthesizer. Crudes are directly usable in downstream bioassays (no purification), enabling rapid validation results to guide next steps.

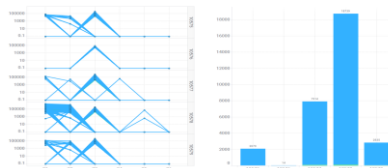
- **Rapid Decision-Making:** Design-synthesis-test cycles completed in **2-4 weeks per round**, enabling go/no-go decisions within **6 weeks**.
- **Cost-Effective:** **3-10 times** more economical than traditional medicinal chemistry approaches.



Case Study: Parallel Peptide Synthesis and ASMS Validation

Peptide DEL screening

Enriched signals / Compounds selection



HTC-peptide resynthesis

48 peptides



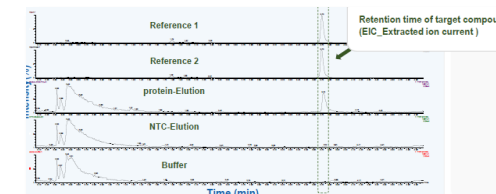
Assay ready plate

Without purification
LC-MS yields

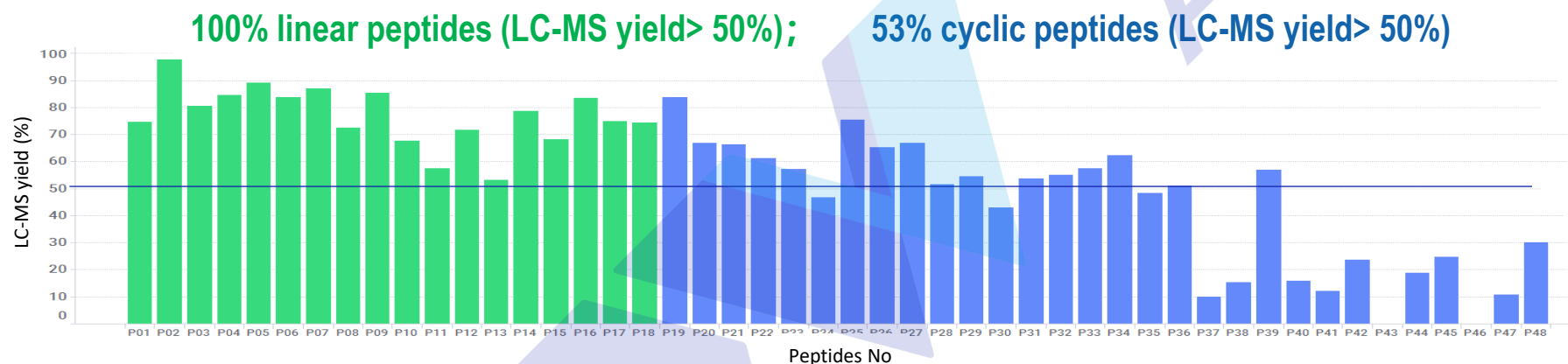


Direct-to-Biology assay

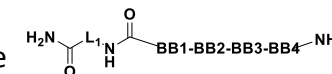
Single peptide ASMS



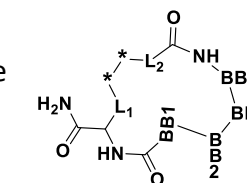
LC-MS yield for synthesized peptides



Linear peptide



Cyclic peptide



✓ Binding affinity validated via the protein-immobilized ASMS method.

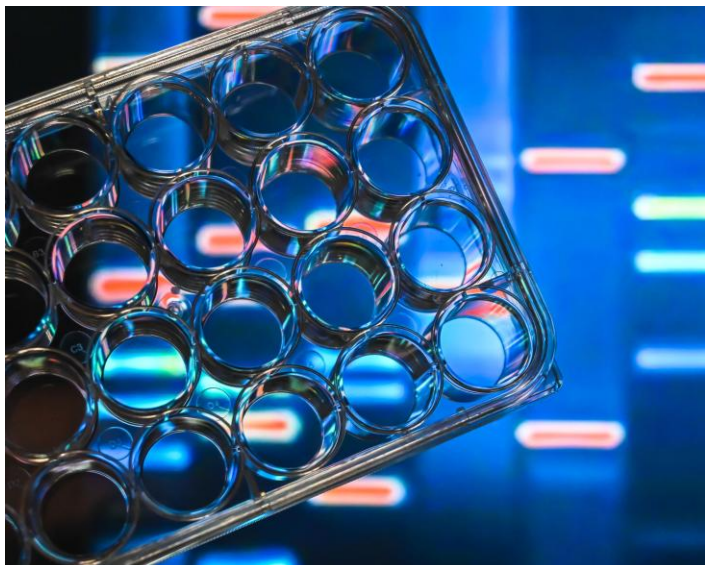
Protein A: 50 % positive rate

Protein B: 70 % positive rate

Integrated Bioassay Platform for Peptide Profiling



	Binding/PPI	Permeability	Stability	Functionality	Specificity/Toxicity
Biophysics	✓				
Biochemistry	✓	✓	✓	✓	✓
Structural Biology	✓				
Cell Biology		✓	✓	✓	✓
ADME		✓	✓		✓
DMPK		✓	✓	✓	✓



A comprehensive spectrum of biology services and solutions, supporting stand-alone and integrated projects, from target discovery to hit finding, lead optimization, candidate selection, and beyond.

- **Comprehensive** discovery and translational biology centers, with **~3,000** experienced scientists and global footprints in 9 sites
- Early discovery screening platform, providing diverse **hit finding solutions** and **high throughput hit optimization solutions** such as DEL/HTS/HCS/ASMS/FBDD*/Display/virtual screening and Direct-to-Biology, supported by informatics and data sciences
- **Thousands** of validated, 'ready to go', *in vitro* assays and *in vivo* models enabling discovery biology for comprehensive target classes, therapeutic areas and modalities
- **Extensive Oncology, Immunology, Infectious Disease, Inflammation, Neuroscience, Rare disease and Metabolic Disease indication**, offering an end-to-end service from discovery, through optimization and into clinical development
- **AAALAC** accredited and **BSL-2** certified on multiple sites
- Pathology and **CAP-certified** FACS capabilities supporting clinical biomarker services

Modalities	Early Discovery	Lead Optimization	<i>In Vivo</i> Pharmacology
• Small molecules • Peptides • Oligonucleotides • Bi-functional molecules, e.g. ADC/PDC/POC/TPD* • Vaccines • Cell Therapy	• New target discovery and mechanistic study • Protein production & structural biology • Screening & hit identification: DEL/HTS/HCS/ASMS/FBDD/Display/VS and libraries for small molecules, covalent, peptides, TPD & macrocycles • High throughput hit optimization: D2B platform	• <i>In vitro</i> biochemistry and cell biology • Cell panel screening • MOA studies • Radiometric assays • Clinical biomarker development and validation	• Comprehensive <i>in vivo</i> disease model collection • Targeted oncology and immuno-oncology • Drug resistance and other novel models of higher translational value • Tumor model database

*DEL: DNA-Encoded Library; HTS: High Throughput Screening; HCS: High Content Screening; ASMS: Affinity Selection Mass Spectrometry; FBDD: Fragment-Based Drug Discovery
ADC: Antibody-Drug Conjugate; PDC: Peptide-Drug Conjugate; POC: Peptide-Oligonucleotide Conjugate; TPD: Targeted Protein Degradation

Contact Us



Business Development Contact

Suk Young Cho Executive Director, Korea BD Head

 sycho@wuxiapptec.com

 +82 31 724 2861

Mahnaz Arjomand Executive Director, US BD Head

 mahnaz_arjomand@wuxiapptec.com

 +1 415 755 3735

Dave Madge Vice President, EU and Israel BD Head

 dave_madge@wuxiapptec.com

 +44 7803 124321

Fumio Itoh Executive Director, Japan BD Head

 fumio_itoh@wuxiapptec.com

 +81 75 315 1044

Marcher Xu Executive Director, China, Singapore and Australia BD Head

 xu_longji@wuxiapptec.com

 +86 185 0210 0621



Customer Service

 wuxiconcierge@wuxiapptec.com

 400-820-0985

 www.wuxiapptec.com



WuXi AppTec website



WuXi Biology website



LinkedIn



WeChat

