DNA-Encoded Libraries Empower Early Discovery of Peptide Drugs and Peptide-Based Delivery Tools

WuXi Biology

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Abstract

Peptide therapeutic discovery has enjoyed a resurgence of interest, particularly for historically "undruggable" targets. However, the de novo discovery of peptide ligands remains a challenge. Phage and mRNA display technologies have a solid track record of providing potent peptide ligands, and we actively employ both these platforms. However, the resulting peptide ligands consist of solely or predominantly natural amino acids, and correspondingly often suffer from low metabolic stability and cell permeability, and fast renal clearance. As an alternative technology, we also employ DNA-encoded libraries (DEL). DEL predominately employs unnatural amino acids, and we have generated hundreds of billions of both linear and corresponding cyclic peptide-like molecules. These DEL macrocycles display a wider range of chemical space than traditional peptide libraries, while simultaneously employing smaller ring sizes (6-9 amino acids in size) and generally more desirable physicochemical properties. The DEL platform also enables the use of diverse cyclization strategies beyond disulfide and thiol-ether bonds, such as the 'click' reaction, which we used to produce our current libraries. In our poster we demonstrate the effectiveness of our DELs for the discovery of macrocyclic peptides including the discovery of i) a 9 nM cyclic inhibitor of the MDM2-p53 interaction, ii) a novel cyclic peptide ligands for PCSK9 which are competitive with MK-0616, and iii) potential tumor cell-specific peptide ligands which are being investigated as targeting moieties to conjugate with oligonucleotides or radioisotopes, to precisely deliver payloads to target tissues followed by internalization.

Peptide Discovery and Hit-to-lead Optimization

■ Schematic of DEL molecules

■ Key Features of Peptide DEL

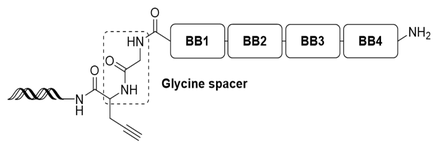
- Amino acid diversity: > 500 natural and unnatural
- N-alkylation: ~ 10% amino acids
- Peptide length: 6 – 9 amino acids
- Cyclization: CuAAC click reaction
- Ring size: 23 – 32 heavy atoms

■ Peptide Optimization Toolbox

- Sequence scanning for primary SAR
- Structure based design from co-crystal
- Docking for peptide-protein interaction
- ML based peptide activity prediction
- Focused DEL and phage display library

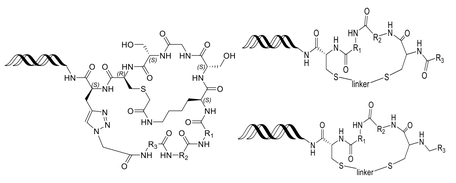
■ Chemical Structures of Peptide DEL

Linear Libraries WXPEP-002 & 003



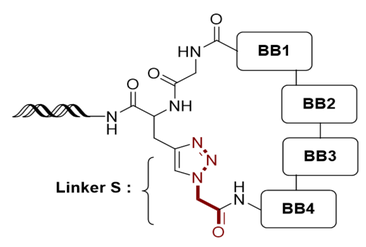
Total diversity: ~95 billion

Bicyclic and oral-focused Library

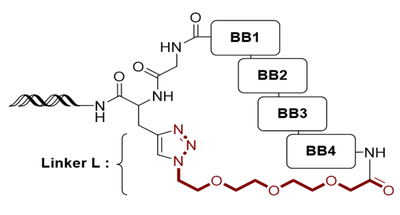


Total diversity: ~42 billion

Cyclic Libraries WXPEP-001 & 004



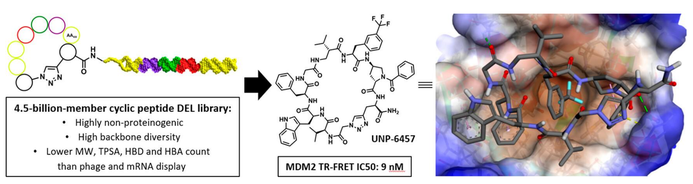
Cyclic Library WXPEP-005



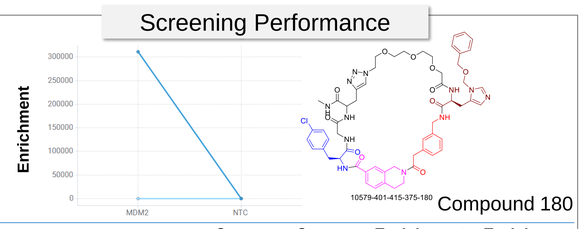
Total diversity: ~156 billion

Case Studies

1. Protein-Protein Interaction: MDM2-p53 Inhibitor



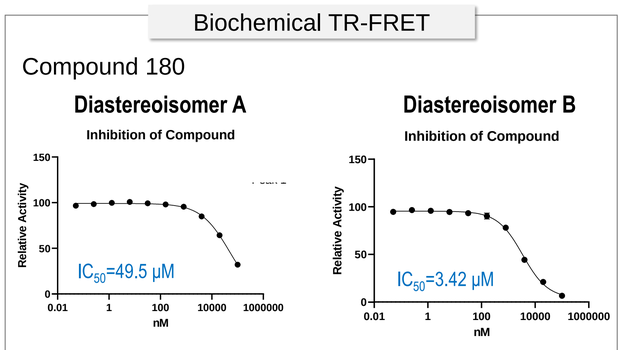
Screening Performance



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

Biochemical TR-FRET

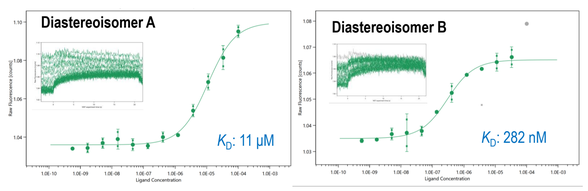
Compound 180



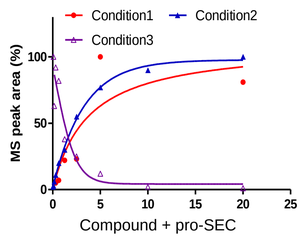
Biophysics MST

Reference: Nutlin-3a, KD=41 nM

Compound 180



SEC-based ASMS

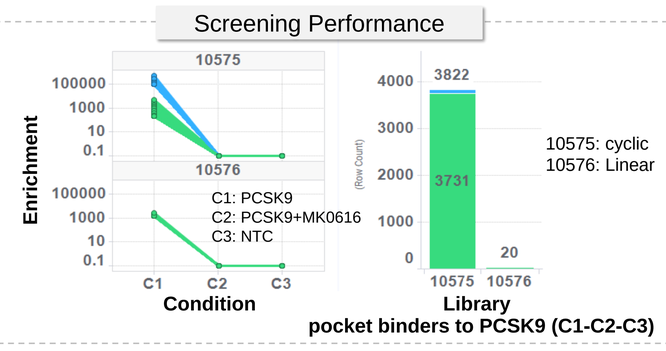


Condition1: Compound 180
Condition2: Compound 260
Condition3: Compound 180 competing by 360

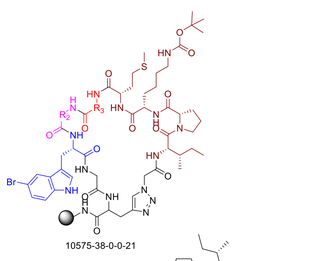
➢**Results:**
✓KD of compound 180: ~3.7uM
✓KD of compound 360: ~1.5uM

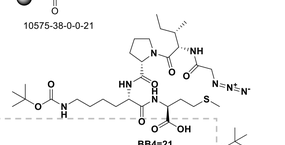
2. Discovery of PCSK9 Inhibitor from WuXi AppTec Peptide DEL

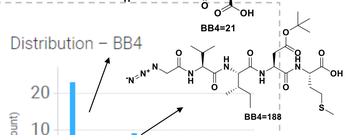
Screening Performance



Potential PCSK9 binder

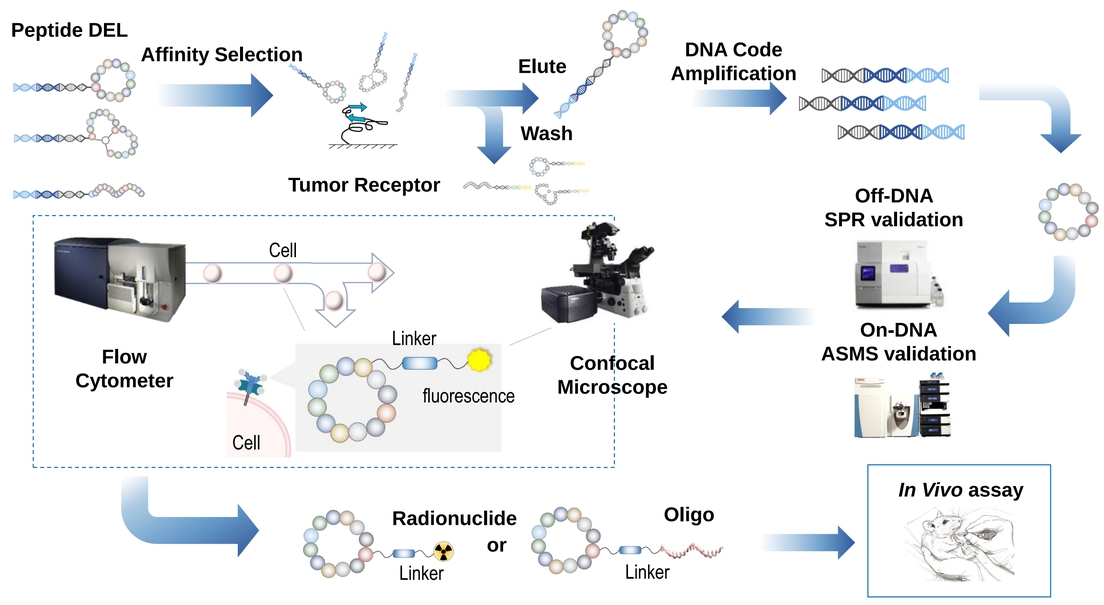




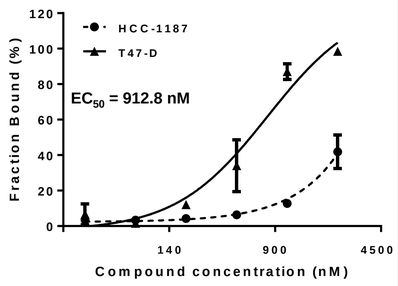


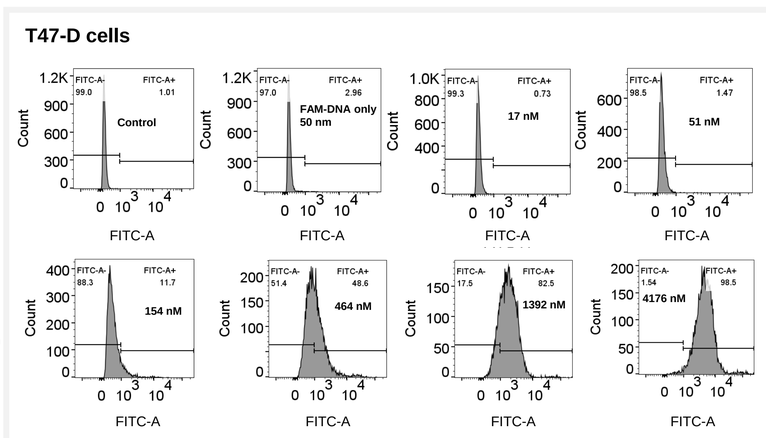
Structure Pattern

3. Discovery of Tumor Cell-specific Peptide Ligands



Synthesis of the tumor-specific radionuclide & oligo conjugate





Note: The specificity of peptide ligands to cells based on membrane protein was demonstrated by flow cytometry in high receptor expression breast cancer cell (T47-D, 90%) and low expression cell (HCC-1187, 10%) under 4 h incubation. FAM-DNA_{20} / tool compound ratio: 1/1; DNA_{20}: 20 base pairs.

Summary

➢ Macrocycles generated from WuXi AppTec peptide DEL can inhibit MDM2-p53 protein-protein interaction. Hits were confirmed by orthogonal biophysical and –chemical assays, and structural biology.

➢ Potential PCSK9-LDLR inhibitors were discovered through DEL screening with MK-0616 as competitor. The structure pattern and SAR information pave the way to hit-to-lead optimization.

➢ DEL screening identified promising peptide ligands binding to tumor-cell specific endocytosis receptors. Their oligonucleotide delivery capacity and endosomal release are being investigated.

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