Intelligent Hit Discovery Platform for Accelerated Cyclic Peptide Radiopharmaceutical Development

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Introduction

We present an integrated hit discovery platform for radiopharmaceutical drug conjugates (RDCs) that synergizes high-throughput biophysical screening (SPR, SPS) with phage display technology to rapidly identify and optimize high-affinity cyclic peptide binders against oncology targets. This platform addresses critical challenges in RDC development by providing a streamlined solution for hit discovery, optimization, and validation.

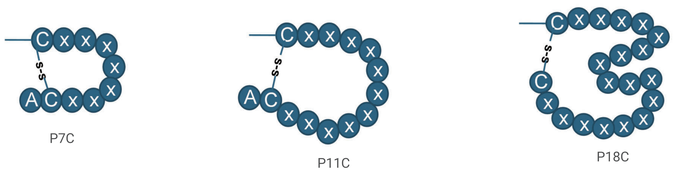
Our methodology employs parallel in vitro biophysical test, phage display library screening. Target validation was achieved through engineered recombinant protein constructs (including PSMA, DLL3 and GPC3) and isogenic cell lines with uniform target expression. Comprehensive evaluation was performed using modular functional assays measuring binding affinity and cellular internalization kinetics.

This platform will significantly accelerate the RDC development pipeline—especially for hit binder identification for neo tumor antigens or other validated surface proteins like GPCRs, transporters—while ensuring optimal tumor targeting and minimized off-target effects.

Keywords: Radiopharmaceutical drug conjugate (RDC), target discovery, cyclic peptide binder, biophysical screening, phage display.

Cyclic peptide phage display library construction and screening

A



B

Library	Capacity
P7C	1.22E+10
P8C	1.41E+10
P9C	2.25E+10
P10C	1.85E+10
P11C	2.32E+10
P12C	1.45E+10
P13C	2.21E+10
P14C	1.32E+10
P15C	4.51E+10
P16C	3.50E+10
P17C	1.60E+10
P18C	3.19E+10

C

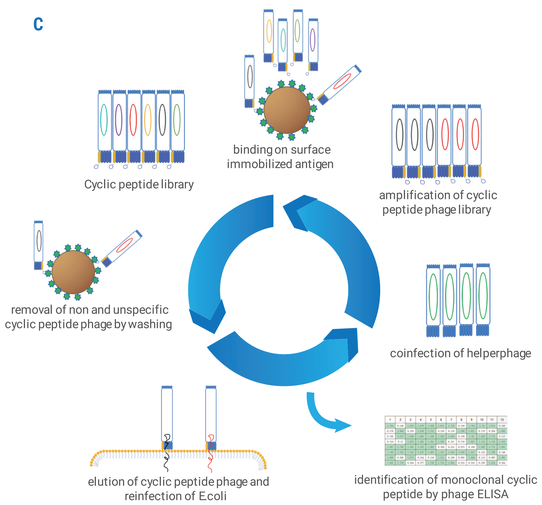


Figure 1.

A Construction of mono-cyclic peptide library in Phages. Cyclic peptides 7–18 aa long (P7C–P18C) were displayed on phage. Library panning against immobilized antigen, helper-phage amplification, and phage-ELISA screening yielded tight-binding monocyclic clones.

B Cyclic peptide library capacity total with **1.53E+11 from 52 different phage libraries.**

C Phage display library screening cascade. A cyclic-peptide phage library was panned against immobilized antigen. After washing away non-binders, helper-phage super-infection was used to amplify eluted phage. Monocyclic peptides were identified by phage ELISA following E. coli re-infection.

Target protein purification and characterization

Figure 2. Post-purification, each protein is verified for stability and purity, then qualified by SPR, SPS and biochemical assays for tight, specific binding to its cognate target before entering binder screening.

Biophysical assay validated target list for binder screening

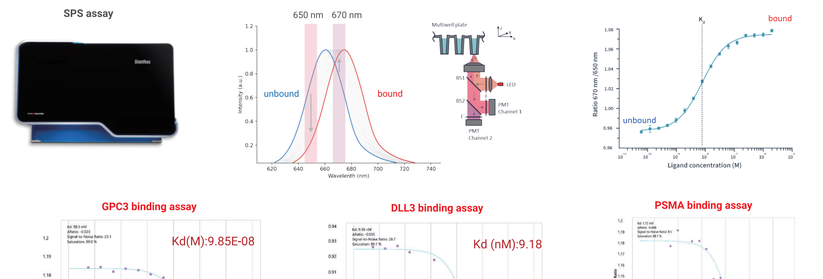


Figure 3. Spectral shift assay (SPS) application in RDC target (e.g., GPC3, DLL3 and PSMA) binder screening comparison with SPR and biochemical assays

Cellular binding and internalization assay profiling

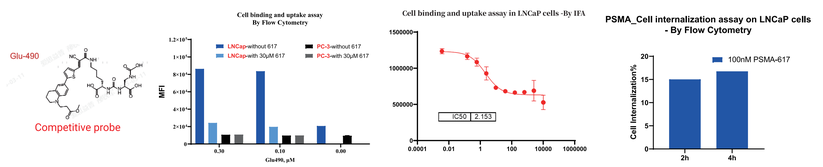


Figure 4. LNCap and PC-3 cells were used to measure compound binding with Fluoscent probe Glu-490 by flow cytometry. PSMA antibody was used to detect internalization of compounds.

Positive clones from Phage display library ELISA assay

P7C ~ P10C

NP01-1 Round 3 positive clone test

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.794	0.149	1.625	1.679	1.589	1.651	1.726	0.159	1.764	1.74	1.674	0.182
B	0.176	1.668	0.105	1.626	1.731	0.138	0.119	1.586	1.631	0.178	0.204	1.805
C	0.186	1.673	1.648	1.605	1.596	1.535	1.604	0.199	1.62	1.628	1.718	0.117
D	0.154	0.12	1.655	1.491	1.581	1.595	0.181	0.158	1.667	1.313	1.118	1.433
E	1.597	1.759	1.392	1.532	1.685	0.169	0.214	0.075	0.108	1.636	1.772	1.981
F	1.746	1.722	1.745	1.637	1.483	1.557	1.583	1.639	1.647	0.125	1.799	0.148
G	1.689	0.208	1.672	0.154	0.134	1.521	0.109	0.095	1.668	0.113	0.095	1.891
H	1.842	1.718	0.264	0.277	1.728	1.753	1.684	0.223	0.232	0.256	1.824	0.264

P11C ~ P14C

NP01-2 Round 3 positive clone test

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.374	2.188	2.309	2.29	2.144	2.091	2.035	2.406	2.211	2.155	2.273	2.035
B	2.359	2.306	2.317	2.434	2.313	2.343	2.029	2.13	1.971	2.007	2.175	2.029
C	2.264	2.208	1.96	2.187	2.021	1.877	0.233	2.033	2.03	2.148	2.108	2.021
D	2.307	2.264	2.057	2.358	2.067	2.034	2.164	2.128	2.117	2.19	2.209	2.065
E	2.479	2.322	0.503	2.094	2.325	2.024	2.026	2.094	2.017	1.755	2.248	2.204
F	2.393	1.975	2.113	2.13	2.1	2.106	2.106	2.117	1.967	2.313	0.166	1.992
G	2.214	2.196	1.894	2.072	2.024	2.222	1.988	0.182	2.113	2.15	2.131	1.96
H	2.142	2.159	2.014	2.131	1.96	2.044	2.053	1.986	1.936	2.048	2.022	2.081

DLL3

NP05 (7-10) Round 3 positive clone test

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.121	0.084	0.091	0.082	0.086	0.108	0.095	0.069	0.076	0.065	0.076	0.109
B	0.067	0.069	0.055	0.065	0.06	0.052	0.057	0.058	0.059	0.066	1.924	0.076
C	0.063	0.047	0.046	0.05	0.065	0.049	0.049	0.052	0.051	0.054	0.053	0.07
D	0.098	0.077	0.075	0.076	0.079	0.08	0.073	0.079	0.076	0.083	0.074	0.092
E	0.047	0.051	0.048	0.055	0.047	0.045	0.052	0.057	0.053	1.61	0.05	0.053
F	0.106	0.093	0.081	1.714	0.071	0.071	0.092	0.069	0.069	0.071	0.066	0.088
G	0.146	0.066	0.061	0.07	0.066	0.061	0.059	0.065	0.065	0.061	0.076	0.088
H	0.273	0.248	0.233	0.217	0.228	0.237	0.221	0.214	0.213	0.196	0.206	0.212

GPC3

NP05-2 Round 3 positive clone test

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.128	0.101	0.096	0.117	0.126	0.097	1.278	0.123	0.154	0.104	0.127	1.501
B	0.114	0.118	0.214	0.099	0.083	0.084	0.088	0.086	0.087	0.109	0.143	0.18
C	0.12	1.53	1.481	0.064	0.074	1.466	0.086	0.094	0.113	1.543	0.139	0.213
D	0.145	0.284	0.099	0.169	0.127	1.481	0.106	0.14	0.115	0.226	0.167	0.191
E	0.118	0.138	0.154	1.472	0.068	1.441	0.11	0.086	0.103	1.606	0.139	0.177
F	0.122	0.119	0.213	0.097	0.094	0.096	0.132	0.295	0.107	0.127	0.154	0.169
G	0.139	0.139	0.09	1.548	0.15	0.126	1.283	0.098	0.104	0.13	1.505	0.193
H	0.27	0.364	0.334	0.298	0.215	0.222	0.242	0.32	0.281	0.335	0.333	0.4

Table 1. Recombinant human DLL3 and GPC3 ectodomains were sequentially challenged with a phage-displayed library of 10- to 17-residue monocyclic peptides (P7C–P14C). After three stringent selection rounds, high-affinity binders were enriched and visualized as discrete green-highlighted color. The DNA encoding these candidate peptides has now been bulk-amplified and is undergoing high-throughput NGS to decode clone diversity, reveal consensus motifs and rank variants by frequency before off-phage affinity confirmation.

Target and assays summary list

Target Name	Human Protein Length	Species
DLL1	AA:Q18-G540	Human
DLL3	1)AA:A27-L492 2)AA:A27-E215 3)AA:V311-A479 4)AA:K352-A479 5)AA:E215-A479 6)AA:E215-K352	Human Mouse Cynomolgus
DLL4	AA:S27-P524	Human
GPC1	AA:D24-S530	Human
GPC3	AA:Q25-H559	Human Mouse Rat Cynomolgus Dog
GPC5	AA:E25-T554	Human
GPC6	AA:D24-S529	Human
HER2	AA:T23-T652	Human
PD-L1	AA:F19-Y134	Human
PSMA	AA:44-750	Human Mouse Rat Cynomolgus Rabbit
CA9	AA:38-414	Human
FAP	AA:26-760	Human Mouse Rat Cynomolgus Rabbit
PARP1	AA:A2-W1014	

Target Name	Protein	SPR	Spectral shift	Cellular assay
Human GPC3	√	√	√	√
Mouse GPC3	√	√	-	√
Rat GPC3	√	√	-	√
Cynomolgus GPC3	√	√	-	√
Dog GPC3	√	√	-	√
DLL3	√	√	√	√
PD-L1	√	√	√	√
Human PSMA	√	-	√	√
Mouse PSMA	√	-	√	-
Cyno PSMA	√	-	√	-
Rat PSMA	√	-	√	-
FAP	√	√	-	√
PARP1	√	√	-	-
CA9	√	-	√	√

Table 2. RDC target protein, biophysical (SPR and SPS) and cellular assays ready-to-use.

Summary

We successfully established a cyclic peptide evaluation system incorporating recombinant proteins and engineered cell lines for both validated RDC targets (DLL3 and GPC3) and prepare the downstream assays ready to screen once the cyclic peptides are synthesized.

Reference

Zhang, J., Rakhimbekova, A., et al. A prostate-specific membrane antigen activated molecular rotor for real-time fluorescence imaging. Nat Commun 12, 5460 (2021).