AI-Accelerated Discovery of B7-H3 Targeted Cyclic Peptide ligands: From Library Design to Preclinical Validation

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Introduction

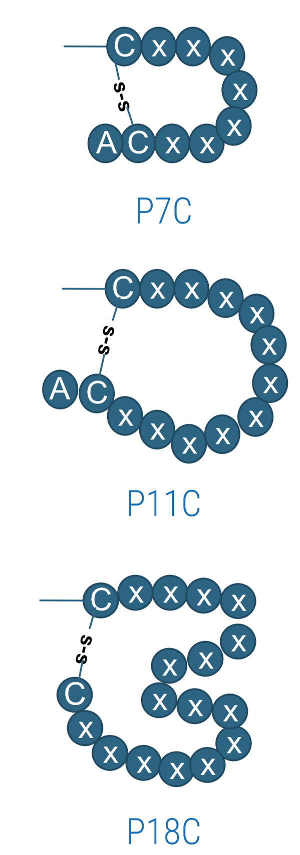
We present an integrated hit discovery platform for B7-H3 macrocyclic peptide binder screening that synergizes high-throughput biophysical screening (SPR, SPS) with phage display technology. State-of-the-art protein structure prediction methods were employed to model the peptide–receptor complex, combined with binding free-energy calculations, thereby enabling the rapid identification and optimization of high-affinity cyclic peptide binders against oncology targets.

Our methodology employs parallel in vitro biophysical assays and phage display library screening. Protein structure prediction tools were also utilized to model the cyclic peptide–receptor complex structure, followed by binding free-energy calculations. The sequences obtained from phage display library screening were ranked and selected based on the Ipae metric and binding free-energy values. Target validation was achieved through engineered recombinant protein constructs (including B7-H3, FAP, EGFR, B7-H4 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.

Keywords: B7-H3, Radiopharmaceutical drug conjugate (RDC), Macrocyclic peptide (MPC), Binder screening, phage display.

Cyclic peptide phage display library construction and screening

A



B

Library	Capacity
N7C	3.37E+09
N8C	1.11E+10
N9C	6.61E+09
N10C	9.20E+09
N11C	1.03E+10
N12C	7.81E+09
N13C	1.07E+10
N14C	6.66E+09
N15C	4.28E+10
N16C	3.39E+10
N17C	1.52E+09
N18C	4.79E+09
Total	1.49E+11

C

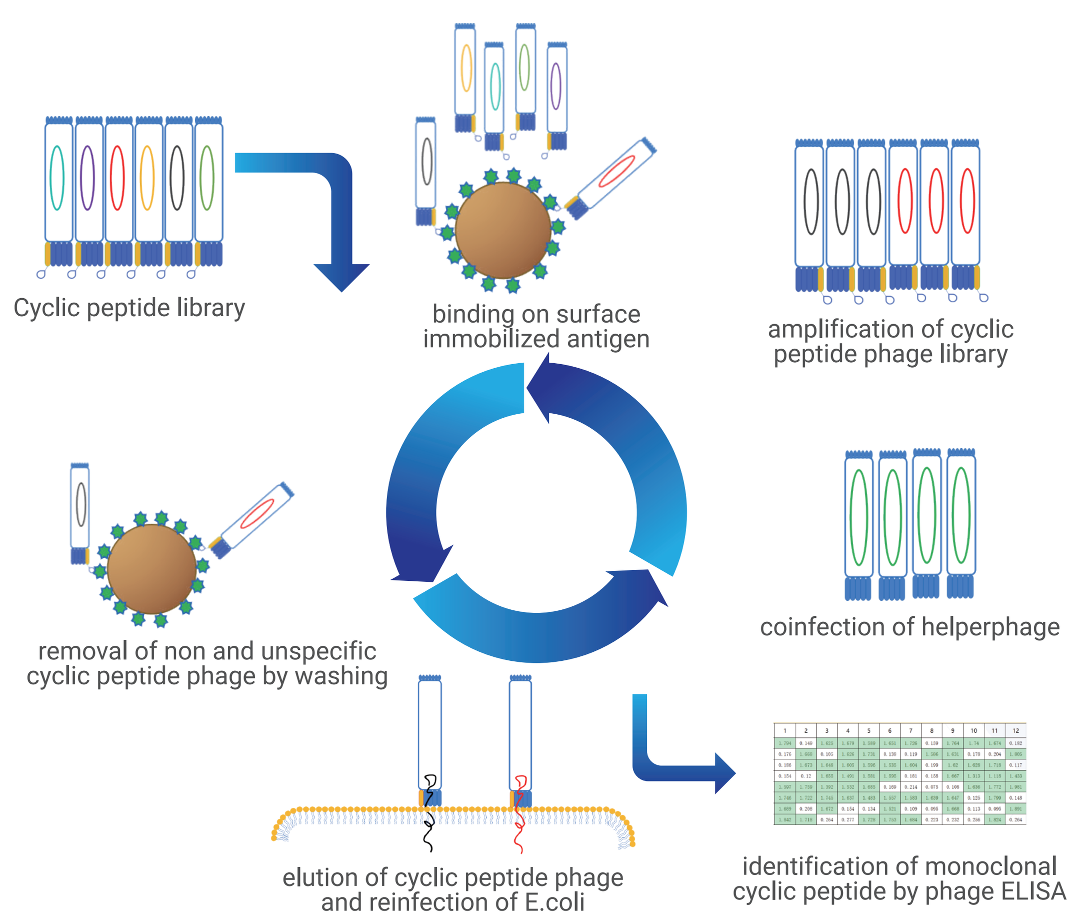


Figure 1. Construction of mono-cyclic peptide library in Phages. Cyclic peptides 7–18 aa long (P7C–P18C) were displayed on phage. Library panning against immobilized antigen, helper-phage amplification, and phage-ELISA screening yielded tight-binding monocyclic clones.
Cyclic peptide library capacity total with **1.49E+11 from 52 different phage libraries.**
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

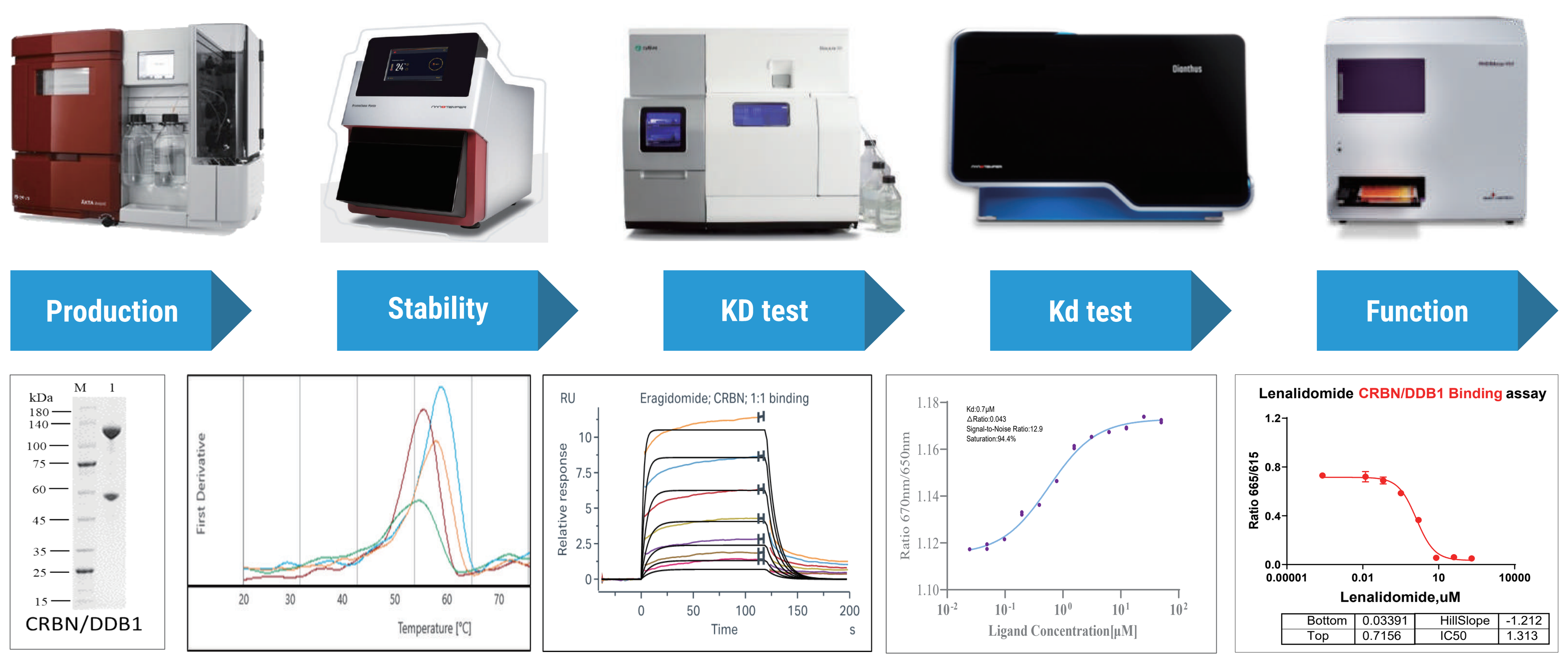


Figure 2. Post-purification, each protein is verified for stability and purity, then qualified by SPR, SPS and biochemical assays for tight, specific binding to its cognate target before entering binder screening.

Positive clones screening and selection

P13C ~ P15C

B7-H3

NP03 positive clone test	1	2	3	4	5	6	7	8	9	10	11	12
A	1.196	1.054	1.36	1.9	1.008	1.15	1.186	1.571	1.611	1.34	1.621	1.454
B	1.976	0.835	1.55	0.516	0.839	1.701	1.932	1.758	1.486	1.568	1.622	1.645
C	2.02	1.71	0.805	1.262	1.259	1.321	1.201	1.468	0.985	1.245	0.981	1.891
D	1.651	1.543	0.736	1.173	1.218	1.534	1.014	0.852	1.698	1.564	0.822	0.767
E	0.63	1.547	1.499	1.324	1.018	1.945	0.16	0.369	0.861	1.146	0.402	1.527
F	0.617	1.002	0.832	1.17	1.64	0.815	0.664	1.631	0.734	1.195	1.053	1.839
G	1.187	0.493	2.04	1.797	1.05	1.427	0.796	1.11	2.095	1.746	1.729	1.788
H	2.228	1.711	1.839	1.137	1.194	1.467	0.472	1.688	1.464	1.772	1.311	1.727

NP03 Negative control test	1	2	3	4	5	6	7	8	9	10	11	12
A	0.174	0.206	0.148	0.202	0.155	0.215	0.171	0.189	0.253	0.184	0.215	0.324
B	0.186	0.143	0.134	0.12	0.156	0.16	0.151	0.159	0.158	0.093	0.187	0.233
C	0.148	0.134	0.121	0.127	0.102	0.119	0.131	0.105	0.117	0.109	0.135	0.189
D	0.214	0.204	0.095	0.122	0.113	0.126	0.124	0.098	0.126	0.157	0.182	0.309
E	0.187	0.164	0.13	0.098	0.119	0.083	0.089	0.105	0.11	0.142	0.172	0.327
F	0.221	0.182	0.123	0.164	0.152	0.126	0.13	0.136	0.131	0.149	0.166	0.283
G	0.227	0.142	0.16	0.177	0.199	0.123	0.148	0.182	0.207	0.173	0.237	0.282
H	0.378	0.381	0.558	0.394	0.496	0.365	0.366	0.328	0.354	0.372	0.555	0.517

Table 1. Recombinant human B7-H3 ectodomains were sequentially challenged with a phage-displayed library of 13- to 15-residue monocyclic peptides (P13C–P15C). 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.

Biophysical assay validated target list for binder screening

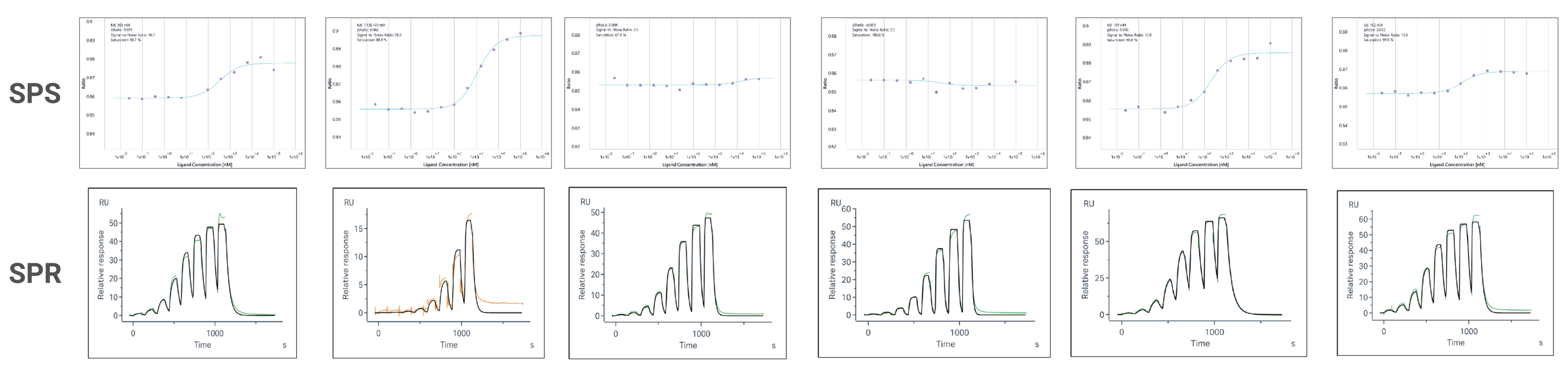


Figure 4. SPS and SPR assay results in RDC target binder screening for B7-H3.

Cyclic Peptide #	Kd from SPS, nM	△Ratio, SPS	KD from SPR, nM	Ipae, Å	MM-GBSA, kcal/mol
1000	360	0.019	176	7.06	-91.89
1001	1130	0.042	100	6.39	-106.93
1002	N/A	0.004	413	6.87	-107.01
1003	N/A	0.003	564	6.52	-100.78
1004	189	0.030	193	-	-85.29
1005	102	0.012	128	8.88	-113.92

Table 2. The summary of biophysical assays and calculated Ipae and MM-GBSA results for all 6 selected peptides.

The same protocol of candidate discovery and evaluation was applied to other validated RDC targets (B7-H4, EGFR, GPC3 and FAP) and new batch of cyclic peptides are selected and synthesized based on the calculated Ipae and binding free-energy.

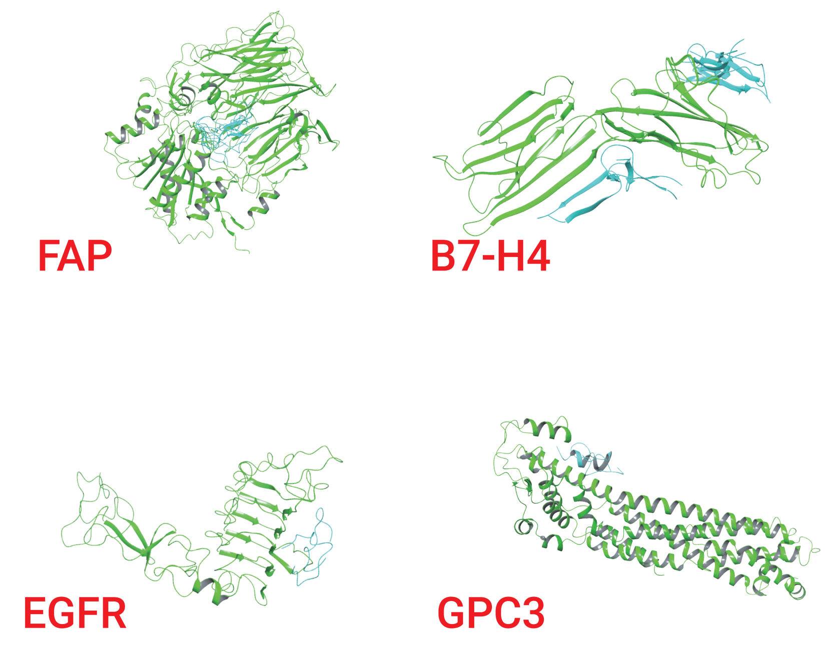


Figure 4. Predicted complex models for new targets (FAP, EGFR, B7-H4 and GPC3). Peptides are colored in teal, and receptors are colored in green.

Receptor	Cyclic Peptide #	Ipae, Å	MM-GBSA, kcal/mol
FAP	F-1000	13.51	-100.46
	F-1001	13.34	-85.03
	F-1002	12.03	-85.41
	F-1003	14.28	-103.37
	F-1004	12.27	-69.48
	F-1005	11.55	-60.86
	F-1006	12.86	-118.32
EGFR	E-1000	9.03	-39.61
	E-1001	6.81	-54.88
B7-H4	H4-1000	14.74	-59.77
	H4-1001	10.68	-56.97
	H4-1002	13.16	-60.40
	H4-1003	8.87	-59.78
	H4-1004	11.58	-54.33
	H4-1005	8.44	-54.89
	H4-1006	8.59	-66.10
	H4-1007	10.17	-56.86
GPC3	G-1000	19.83	-77.36
	G-1001	24.76	-73.95

Table 3. The summary of calculated Ipae and MM-GBSA results for 19 cyclic peptides after the phage display library screening process.

Summary

We successfully established a cyclic peptide discovery and evaluation system incorporating recombinant proteins and engineered cell lines for validated RDC targets (B7-H3, B7-H4, EGFR, GPC3 and FAP) 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).