PEPTIDE MICROARRAY PLATFORM FOR HIGH-THROUGHPUT DISCOVERY AND OPTIMIZATION OF PROTEIN-BINDING PEPTIDES

P420

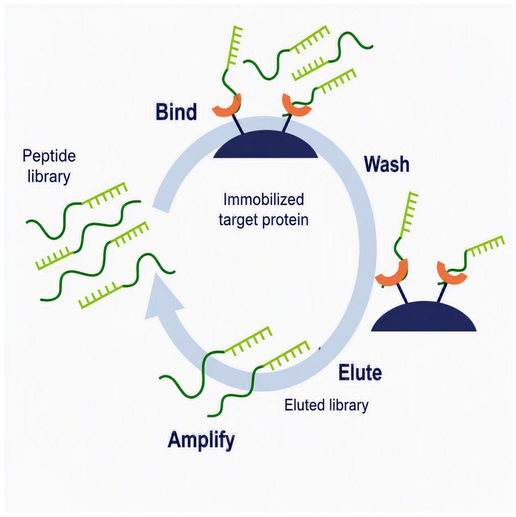
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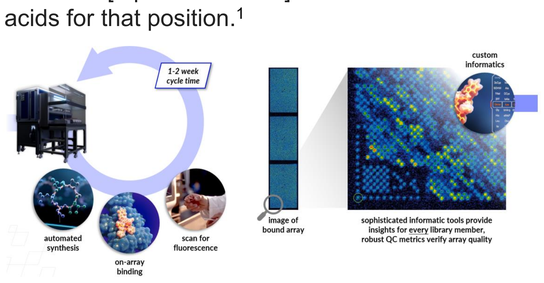
DISPLAY

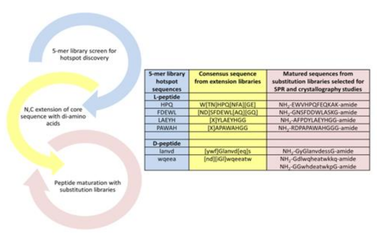
- Both phage and mRNA display facilitate the screening of highly diverse peptide libraries (from 10^9 to 10^{13} peptides) to identify peptides that bind to a target protein of interest.
- Through multiple rounds of iterative binding, washing, and amplification, high-affinity peptides are enriched.



RATIONAL PEPTIDE ARRAY DESIGN

- A schematic representation of the combined stepwise evolution and rational peptide array design approach.
- The table shows L- and D-peptide sequences (shown in uppercase and lowercase, respectively) selected at different steps of streptavidin binder evolution by using 5-mer, extension, and substitution libraries. Residues shown in [square brackets] are alternative amino acids for that position.[1]

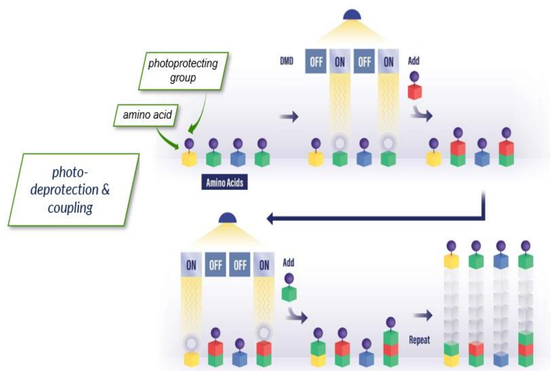




5-mer library hotspot sequences	Consensus sequence from extension libraries	Matured sequences from substitution libraries selected for SPR and crystallography studies
L-peptide		
HPQ	WITN[HPQ]NFA[EGE]	NH$_2$-EWVHPQFEQKAK-amide
FDEWL	IND[SYDEWL]AQ[GSD]	NH$_2$-GNDFDDWLASKG-amide
LAEYH	[KTYLAEYHGG	NH$_2$-AFPDYLAEYHGG-amide
PWAAH	[KJAPAWAHGG]	NH$_2$-RDPAPAWAHGGG-amide
D-peptide		
lamvd	[ywfiSGlamvd[eq]s	NH$_2$-GyGlamvdessG-amide
wqeea	[nd][Gf]wqeeatw	NH$_2$-GdwgheatwkkG-amide
		NH$_2$-GGwhdeatwkyG-amide

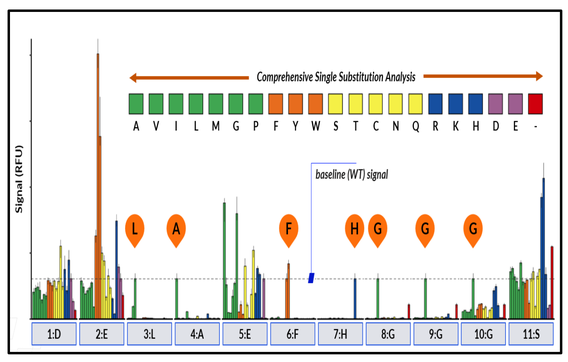
PEPTIDE MICROARRAYS

- Peptide microarray platform enables the synthesis of millions of user-defined peptides on a glass slide using patterned UV light and amino acids with photo-protecting groups.
- After on-array binding of fluorescently labelled protein, the slide is scanned for fluorescent signal, and the intensities are mapped to the identity of peptide synthesized in each feature.

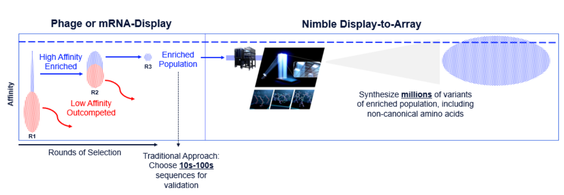


ARRAY DATA READOUT & ANALYSIS

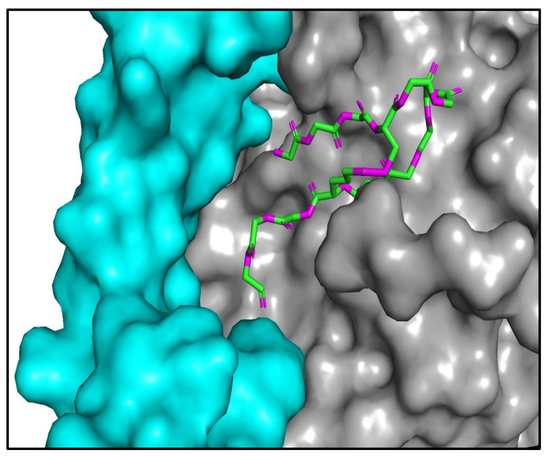
- Comprehensive amino acid mutational scanning on the array is used for high-throughput validation of potential peptide binders from display selections.
- The baseline fluorescent signal is used to identify peptides that bind the target protein while the signal of the single amino acid mutants pinpoints residues critical for protein binding.



TOGETHER: DISPLAY AND ARRAY



One such optimized peptide identified via phage display has been matured with non-natural incorporation of amino acids on array for Protein X, to identify 8 nM binder. The image below depicts peptide co-crystallized with ligand (Grey) followed by overlay on ligand-receptor (Grey-Cyan) structure. Peptide side chains are removed from this image to not disclose the sequence identity of the peptide.



EXAMPLE OF A WORKFLOW

DISPLAY

Find enriched binders

↓

ARRAY

Validate and map sequence determinants

↓

CHEMISTRY

Introduce non-natural residues or cyclization

↓

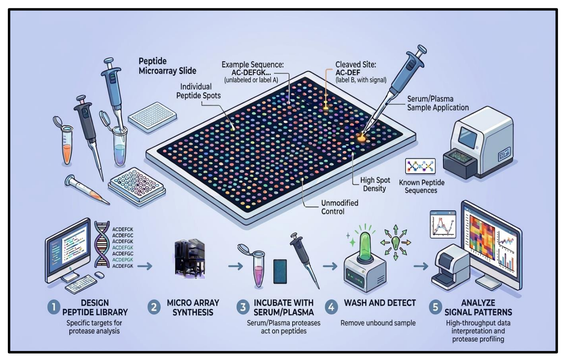
MEASURE

Track affinity, specificity and stability

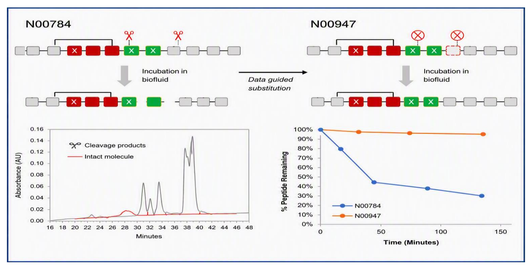
Integrated iteration accelerates progression from hit identification to optimized peptide properties.

PEPTIDE STABILITY STUDIES

The image below is generated using go/AI tool in AbbVie. The Step1 to 5 are Design Peptide Library, Make Microarrays, Incubate Biofluids, Detect, Analyze. [2]



P001 is the reference sequence that has 2 cut sites. Arrays are deployed to identify mutations in P001 that tolerated the biofluid cut sites. This resulted in identifying P002. The stability improvement is visually depicted below where % Peptide Remaining is measured against Time.



AI/ML DISCOVERY HITS ON ARRAY

Peptide sequences designed using AI/ML tools are explored on array to identify streptavidin binders.

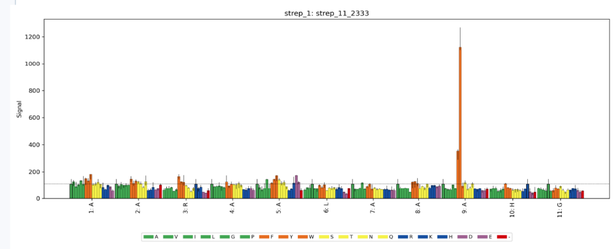


Fig 1: Initial ML-designed peptide displayed mild specificity for wild-type sequence

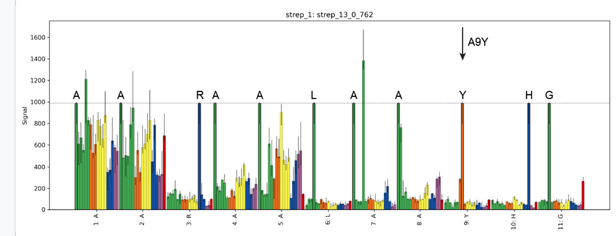


Fig 2: A single array-suggested point mutation drastically improved peptide binding signal and specificity for wild-type sequence

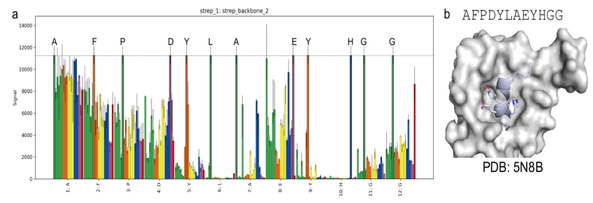
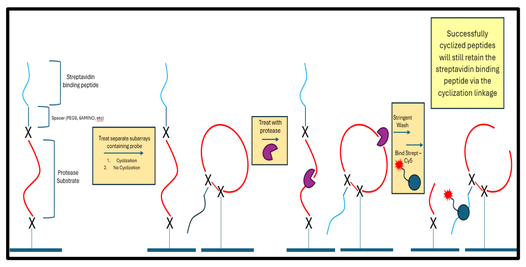


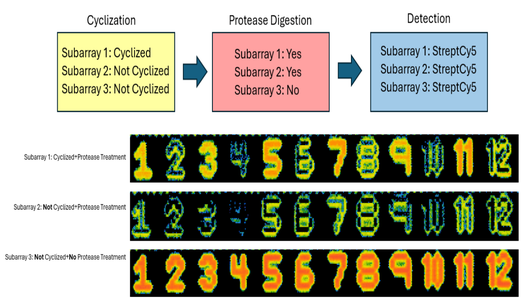
Fig 3: a) Mutation scan plot of known streptavidin binder, AFPDYLAEYHGG b) The ML-designed peptide overlaps well sequence- and specificity-wise with known helical streptavidin binder

METHOD DEVELOPMENT: MACROCYCLIZATION

The macrocyclization on the array is tested using Streptavidin binding peptide and known protease substrate where X represents the cyclization monomers in odd-numbered sub-arrays and non-cyclization monomers in even-numbered sub-arrays.



Example Data:
Numbers 1,3,5,7,9,11 → protease substrates flanked with cyclization monomers ("**can** be cyclized")
Numbers 2,4,6,8,10,12 → paired linear protease substrate controls in which the cyclization monomers are replaced with non-cyclizing monomers ("**cannot** be cyclized")



TAKE-HOME MESSAGE

Display finds hits. Arrays explain, optimize and de-risk them

- **Expanded validation across thousands of sequences**
- **Single-residue resolution for affinity and specificity**
- **Rapid testing of non-natural amino acids and cyclization**
- **Direct integration with AI/ML-designed peptide libraries**
- **Parallel optimization of binding and stability**

Display hit → Array optimization → 8 nM binder

CONCLUSIONS

1. **Peptide microarrays substantially expand post-display validation depth.**
2. **The same platform supports affinity maturation, specificity mapping, stability optimization and chemistry development.**
3. **Array-guided point mutations can produce large improvements in binding signal and specificity.**
4. **AI/ML-designed peptides can be tested and refined experimentally at scale.**
5. **Display and array technologies form a complementary discovery and optimization engine.**

High-throughput arrays turn sequence-level experimental data into clear next-design decisions.

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2. Salisbury CM, Maly DJ, Ellman JA. Peptide microarrays for the determination of protease substrate specificity. Journal of the American Chemical Society. 2002;124(50):14868–14870.

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