High Throughput Discovery and Optimization of Peptide Target Binders with Peptide Display and PEPperCHIP® Peptide Microarrays

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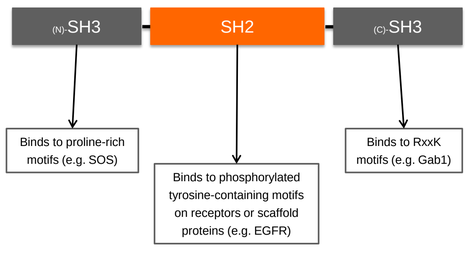
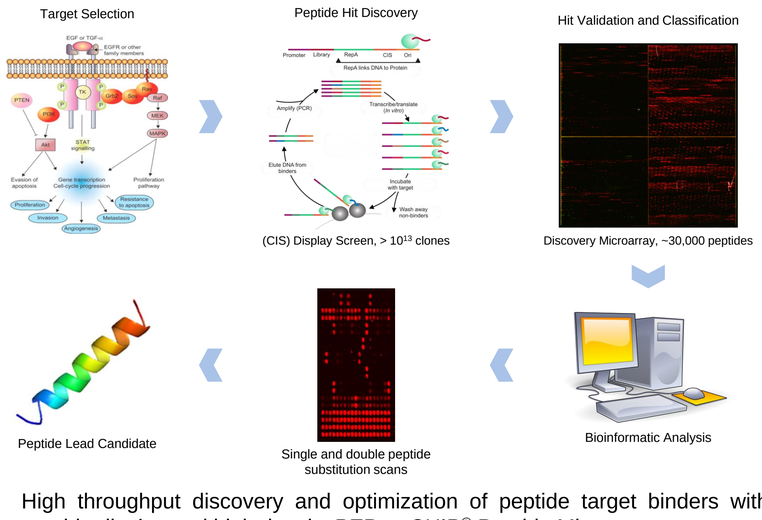
Abstract

We present a new combined approach of peptide display and peptide microarray technologies for the fast and highly efficient development of peptide target binders. In this case study, we discovered and optimized high affinity peptide binders for Grb2, a target protein of the EGFR pathway. 3,796 peptide hits could be identified by an initial CIS display screens performed by Isogenica Ltd. All hit peptides were printed on PEPperCHIP® Peptide Microarrays for hit validation by PEPperPRINT. 12 selected peptides with highest spot intensities were further optimized by single substitution scans identifying a KPLPXXP core signature in 9 of top 12 hits. The core motif was validated by bioinformatics analysis using the MEME Suite and enabled the identification of the same core motif in son of sevenless homolog 1 (SOS) and tyrosine protein kinase BTK as possible Grb2 interaction partners in the EGFR pathway. A second high throughput peptide optimization of 2 selected top peptides was done by double substitution scans with 26,836 peptide variants of the starting motifs including non-natural D-amino acids. Thereby the fluorescent intensity upon Grb2 binding could be increased 6 fold compared to the initial peptide display hits. Selected optimized peptide binders were further investigated by fluorescence polarization in solution and revealed binding affinity in the nanomolar range, 150 times higher than the native SOS peptide.

Workflow and Grb2 as Target Protein

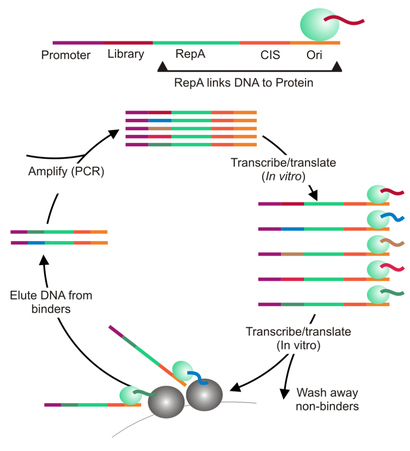
Grb2 is essential for multiple cellular functions e.g. in cancer. The target mediates protein-protein interactions in the EGFR/ HER2 signaling pathway and consists of three interaction domains.





High throughput discovery and optimization of peptide target binders with peptide display and high density PEPperCHIP® Peptide Microarrays.

Hit Discovery: CIS Display Screen



CIS Display:

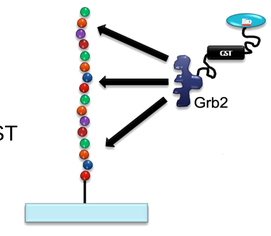
- dsDNA codes for peptide-RepA constructs
- stable peptide-RepA/dsDNA complexes
- libraries of >10^{13} peptides

Peptide Libraries:

- Gab1 motif-based 7R2KP3: $X_7RX_2KPX_3$
- Gab1 motif-based 3R4KP5: $X_3RX_4KPX_5$
- random linear Tri16: X_{16}trimer-cys
- random linear Tri20: X_{20}trimer-cys

Selection Assay:

- biotinylated GST-Grb2
- four rounds in the presence of GST

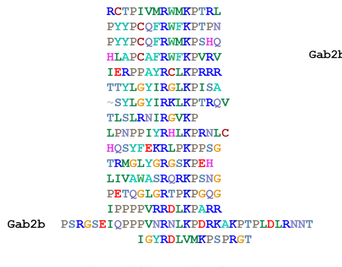


isogenica discovery services for biologics

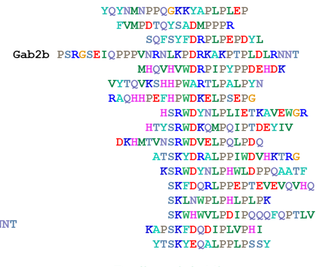
Next Generation Sequencing

- generation of ca. 100,000 DNA sequences for each selection output
- DNA sequences cropped, translated and clustered
- generation of 1,000's of peptide sequences around motifs

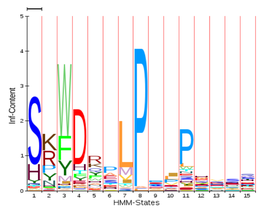
Motif-based libraries



Random linear libraries



Main motif



➢ 3,796 peptide hits for PEPperCHIP® Peptide Microarrays screening

Hit Validation and High Throughput Optimization with PEPperCHIP® Peptide Microarrays

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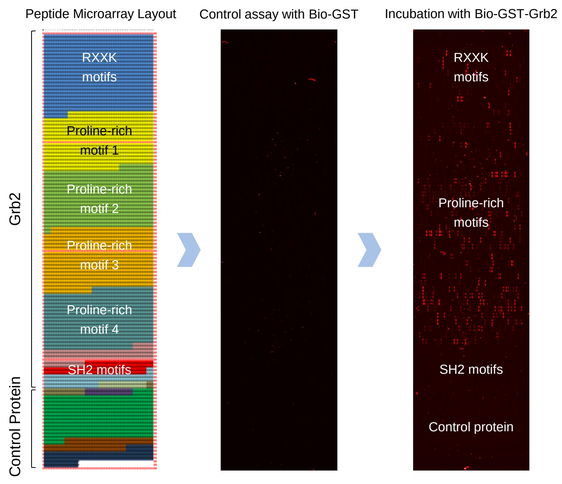
1. CIS-Display Hit Validation

Array content:
- 3,796 selected peptides from CIS Display screen clustered in different motif groups
- control peptides

Assay:
- Biotin-GST-Grb2 with Streptavidine-DyLight680

Outcome:
- 15% of the initial CIS Display hits could be verified by peptide microarray analysis

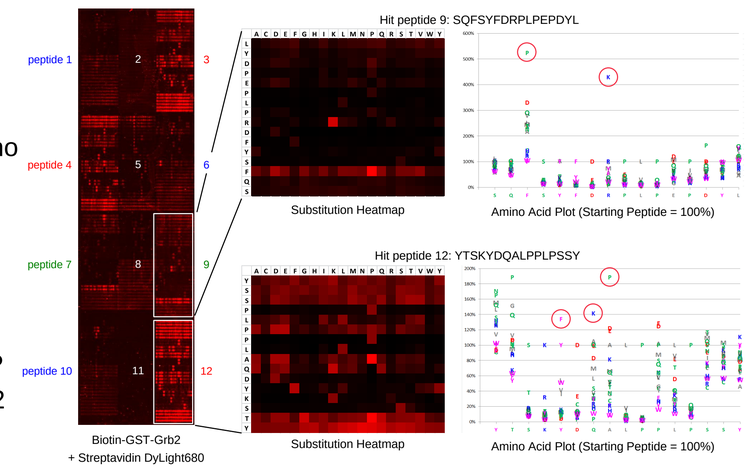


2. Hit Single Substitution Scan

Array content:
- substitution of each amino acid position with 20 L-amino acids of 12 peptide hits

Assay:
- Biotin-GST-Grb2 plus Streptavidine-DyLight680

Outcome:
- identification of a KPLPXXP core signature in 9 of top 12 hits



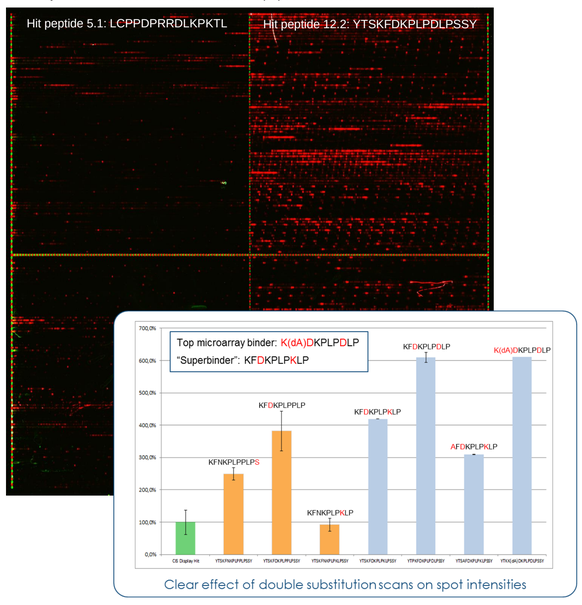
3. Double Substitution Scan

Array content:
- substitution of two amino acid positions at once with 20 L- and D-amino acids dK, dA and dE

Assay:
- Biotin-GST-Grb2 plus Streptavidine-DyLight680

Outcome:
- fluorescence intensity of "super binder" peptide is 6 fold higher than original CIS Display hit

Array with 2 x 26,836 variants of each hit peptide, incubation with Bio-GST-Bio

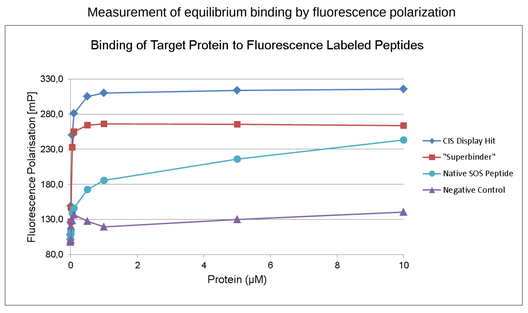


4. Affinity Determination

Samples:
- soluble peptides including "super binder", CIS Display hit, native SOS binder and negative control peptide

Assay:
- monitoring protein binding to fluorescence-labeled peptides by fluorescence polarization

Measurement of equilibrium binding by fluorescence polarization



- novel peptides with nanomolar affinities
- clear optimization by double substitution scans
- >150 fold higher affinity than SOS peptide

Peptide	KD (nM)
CIS Display Hit	29
"Superbinder"	12
Native SOS Peptide	2000
Negative Control	no affinity

Outlook

- affinity determination of other top binders
- fusion of selected top hits with cell penetration peptide
- *in vitro* assay with cellular system to block Grb2 and/or the pathway

Benefits

- straightforward peptide display hit validation and classification by peptide microarray
- linear and cyclic peptides available
- first economic microarray screening of >25,000 peptide variants

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