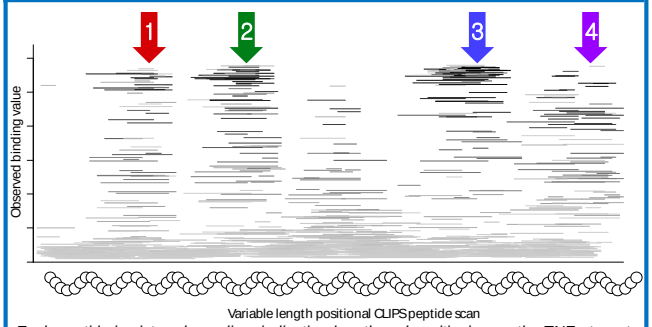
Case report

Conformational Protein-Protein Interaction Mapping of Enbrel®

Objective

The CLIPS Epitope Mapping technology has been shown an effective tool for mapping of conformational and discontinuous epitopes of therapeutic antibodies. This case study demonstrates that the technology can also be used to identify the interaction site between hormones and cell-bound receptors.
Etanercept (Enbrel®) is a chimeric TNFα receptor that is used to treat autoimmune disease by interfering with TNFα (tumor necrosis factor). The interaction between between Etanercept and the TNFα trimer was studied in full detail through the CLIPS Mapping technology.

1. CLIPS variable length positionsal scan

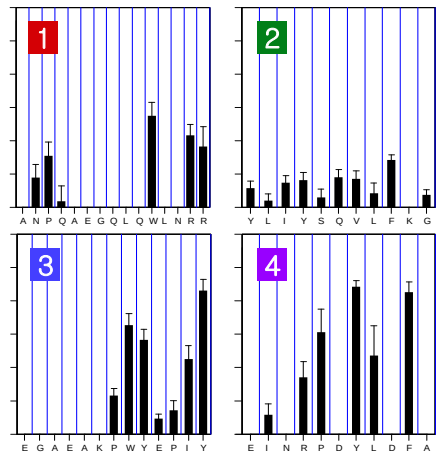


Each peptide is pictured as a line, indicating length and positioning on the TNFα target. Y values indicates binding strength. Colors indicate confidence level of binding.

Method and Results

A set of 8000 CLIPS peptides was synthesized, varying in length from 5 to up to 35 residues and carrying specific disruptive mutations. ELISA analysis identified four binding regions (Fig 1). Validation of these candidates at a single-residue level identifies specific binding residues for areas 1,3 and 4, but no specific binding for area 2 (Fig 2). Visualisation of the identified critical residues from area 1,3 and 4 onto the TNFα receptor bound to the TNFα trimer, modelled through the homologous crystal structure of TNFβ with TNFβ receptor, confirmed that these residues are indeed binding to TNFα receptor, whereas area 2 is not in proximity and may be a non-specific interaction.

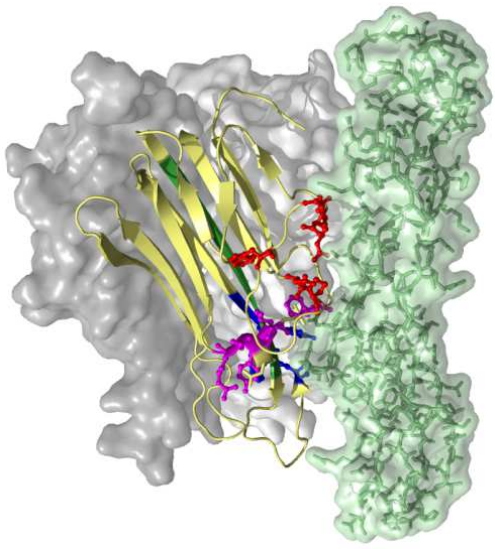
2.Single-residue fine-mapping



Verification of 4 identified binding regions. Each bar indicates contribution to binding per specific residue.

Conclusions

CLIPS protein-protein interaction mapping identified the binding site of the TNFα trimer on the TNFα receptor. Again, the CLIPS technology has proven a powerful tool to study complex interaction sites of proteins. Originally, the CLIPS technology was developed to map conformational epitopes of antibodies. Now it was also used to map interaction sites of another complex protein-protein pair.
The CLIPS technology also shows great promise to study even more complex protein-protein interactions such as between chemokines and their respective GPCR´s, or between peptide snake toxins and ion-channels.



The benefits of CLIPS Protein-Protein Interaction Mapping

- Works for extracellular domains of cell-bound receptors
- Unrivalled single residue resolution
- Re-usable arrays for multiple screenings
- Solid support for patent claims and regulatory filings
- Applicable with very long peptides

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Technical information CLIPS Precision Epitope Mapping

Peptide synthesis	FMOC chemistry. Maximum peptide length over 40 residues. All amino acids, including D-amino acids and non-natural amino acids.
Capacity	Ten custom high-throughput parallel synthesis robots, each 10.000 peptides per run.
Peptide library format	Proprietary 'Minicard' format with solid phase-bound peptide constructs in 455 microwells. Surface chemistry: proprietary polymeric graft optimized for low non-specific binding and high peptide construct loading.
Combinatorial library complexity	Matrix analysis, e.g. 50 x 50 = 2.500 double loop T3 CLIPS™. All matrix combinations within 40-mers possible. All overlapping single loops, usually 15 - 20-mers. All overlapping peptides of a protein, usually 15 - 20-mers. Full positional scan libraries of all epitopes.
Spatial construct complexity	Single loops on T2 CLIPS. Double loop combinations on T3 or 2 x T2 CLIPS. Sheet-like T2 CLIPS, helix-like T2 CLIPS. All loop structures with 2-6 cysteines and 1 or 2 CLIPS.
Peptide library reusability	At least 20 times, but up to 100 depending on the samples. Library storage and re-use up to years.
Binding detection	Binding of the antibodies to the CLIPS peptides is determined in an ELISA. The resulting color in each well is quantified with a CDD camera.
Binding detection sensitivity	Optimized for epitope mapping, down to $K_d=10^{-3}$
Required material and information	100 µl polyclonal serum or 100 µg antibody Linear sequence of target protein.
Project run-through time	Priority 1.5 months, Standard 3 months.
Reporting	Binding values of all peptides are quantified and stored in the PepLab™ database. A full report is provided including details on binding and specificity for each residue, optimized for registration, regulatory,and/or IP purposes. Full support is offered for IP generation and publishing.

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