Case report

Conformational Epitope Mapping of Flu Virus Antibody

Mapping of the FI6 epitope through Helical Coiled Coil Pepscan

Objective:

FI6 is a monoclonal antibody that potentially neutralizes all influenza viruses. To reveal the molecular basis of this pan-influenza cross-reactivite antibody, we fine-mapped the conformational epitope recognized by FI6. (*Corti et al, Science 2011*)

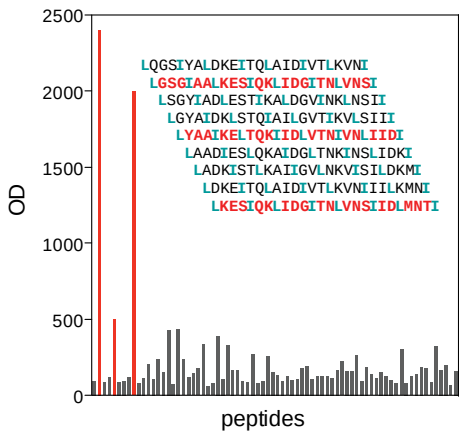
Method and Results:

26-mer peptides derived from the helical HA2 were synthesized at Pepscan. In order to maintain the helical coiled coil conformation every 4th and 7th position of each 26-mer HA2 peptide was replaced by an Ile and Leu respectively. The Pepscan peptide array was screened with monoclonal antibody FI6.

The figure on the right shows the sequences of the first 9 peptides. Blue residues correspond to position 4 and 7 that have been replaced by Ile and Leu. Red sequences correspond to reactive peptides. The bottom sequence line shows the shared residues (in red) of the high binding peptides that contribute to FI6 binding.

The mapping was later confirmed via X-ray diffraction analysis of FI6 bound to HA2 (see below).

Pepscan on coiled-coil peptides

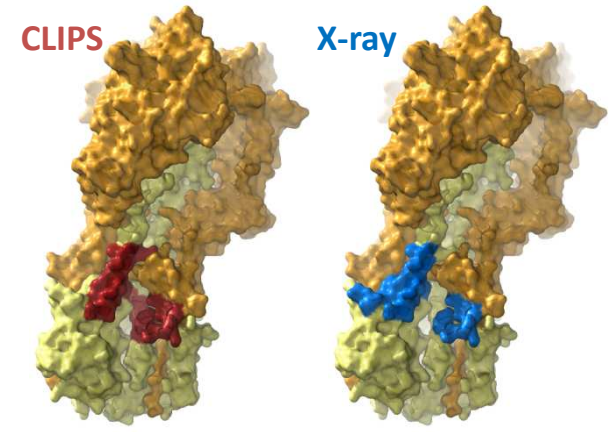


Conclusion:

The coiled coil Pepscan epitope mapping technology has proven to be a powerful tool to study complex conserved epitopes on helices. This helix-stabilizing Pepscan technology is widely applicable for broadly neutralizing antibody selection as well as vaccine development.

Furthermore, in addition to the coiled coil, Pepscan offers a wide range of other structure-inducing scaffolds. These are used to reconstruct complex epitope composed of helices, sheets, loops and combinations thereof. In this way the full range of epitopes of neutralizing antibodies, but also those recognized by therapeutic antibodies or antibodies from sera of cohorts of patients, can be studied in exact detail through Pepscan.

CLIPS epitope mapping vs. X-ray.



The benefits of Pepscan Helical Coil Epitope Mapping

- Works for helix-type epitopes
- Discontinuous, conformational, and linear
- No prior structural information needed
- Applicable to monoclonals and polyclonal sera
- Re-usable arrays for multiple screenings
- Comparative mapping of sets of samples
- Single-residue 3D resolution
- Solid support for patent claims and regulatory filings

This work was performed in a collaboration with Humabs Biomed SA and published by Corti et al, Science 2011:333:850-856.

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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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