Case report

Epitope fine mapping: revealing fine details of anti-HIV-1 F425-B4e8

PEPSCAN

Characterizing the epitope in full detail using full substitution analysis

Objective:

F425-B4e8 is a broadly neutralizing anti-HIV monoclonal antibody that stands out because it recognizes the V3 variable loop region on the gp120 subunit of HIV-1 virus. The V3 loop is a variable region with high degree of conformational flexibility. To date it is known that V3 loop mediates coreceptor binding thereby assisting in the entry of HIV-1 virus. Deep understanding of recognition mechanisms of broadly neutralizing antibodies would not only extend our knowledge on HIV-1 entry, but also may inspire new immunogen designs for vaccine development. In this study HiSense linear mapping with subsequent full replacement analysis was used to examine the fine features of the F425-B4e8 epitope.

Results:

A library of all overlapping linear peptides was designed based on the sequence of gp160. Monoclonal antibody F425-B4e8 was applied under high stringency conditions. Avid binding was recorded on a series of overlapping peptides resulting in the core epitope sequence HIGPRAFY (top left).
Using the results of linear mapping the sequence RKRIHIGPGRAFYT was used to generate a comprehensive series of substitutions, where each position of the epitope is replaced by all other proteogenic amino acids (schematic on top right). Binding of F425-B4e8 to each epitope permutation was recorded and compared to that of the native sequence (bottom right). Results of the experiment show that $_{311}$IGPGRAF$_{317}$ are essential for the binding as most if not all substitutions (R315) diminish the antibody binding.

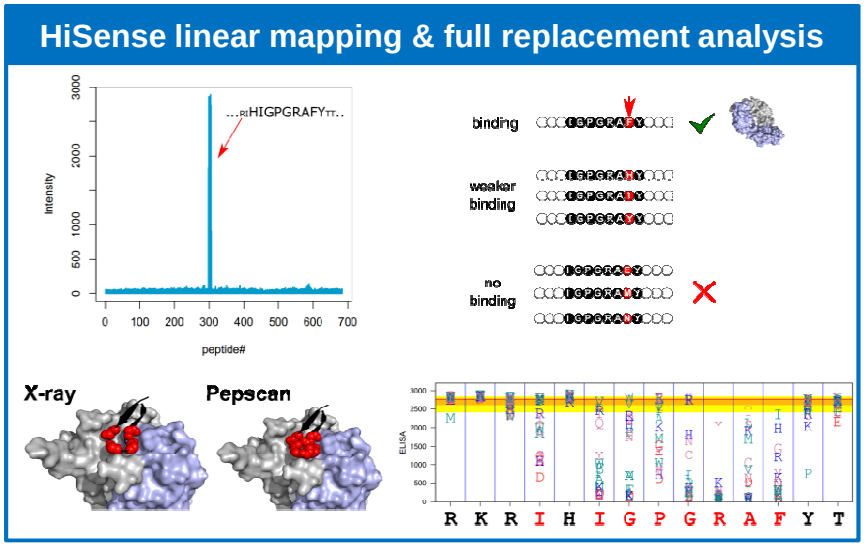

Figure 1: *Initially, the linear epitope for antibody F425-B4e8 was mapped using a HiSense linear epitope approach. Binding profile is shown on the top left panel. To reveal fine features of the antibody binding a full replacement analysis was used, as outlined in the top right panel. In short, such analysis allows identification of essential for the binding residues and those, which provide context for the binding. On the bottom right panel is a letterplot that shows how mutation of positions $_{311}$IGPGRAF$_{317}$ diminishes antibody binding, while replacements of other residues are mostly tolerated. Agreement of the results obtained from Pepscan analysis and X-ray crystallography is visualized using 3D coordinate file 2qsc.pdb on the bottom left panel.*

Most of replacements of I309 significantly decrease the binding, but do not fully abolish it.
These Pepscan results are in agreement with the contact surface identified by crystallography in the co-crystal (2qsc.pdb; bottom left), but the peptide based approach allows finer determination of the paratope tolerance.

Conclusion:

Pepscan HiSense linear arrays allow multiple testing of panels of samples and provide detailed information on the epitopes. Results of peptide-based epitope mapping are in a good agreement with those obtained with X-ray crystallography and can deepen the insights into the mechanism of binding.

The benefits of CLIPS Precision Epitope Mapping

- Works for all types of epitopes
- Discontinuous, conformational, and linear
- Applicable to all kinds of target proteins
- Soluble as well as membrane integrated proteins
- Unrivalled single residue resolution
- Solid support for patent claims and regulatory filings
- Re-usable arrays for multiple screenings
- Comparative mapping of sets of samples

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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	Five 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 $K_d=10^{-3}$ M
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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