Case report

Precision Epitope Mapping of Human anti-SIRPα

Revealing the discontinuous epitope of human anti-SIRPα in full detail

Objective:

Anti-SIRPα is a monoclonal antibody that specifically recognizes recombinant human Signal Regulatory Protein Alpha (SIRPα) and has poor or no cross-reactivity with variants of human recombinant SIRPβ. SIRPα is a receptor of CD47. Binding of CD47 prevents cytokine production.

Challenge:

The epitope of an anti-SIRPα antibody cannot be mapped with linear peptides.

Results:

Pepscan used its competence in CLIPS chemistry to design and synthesize a comprehensive library of combinatorial peptides based on the sequence of SIRPα by introducing structural constraints in arrayed peptides. Heatmap analyses showed that only peptide chimeras containing both YYAVKERKGSPDDVE and GRELIYNQKEGHEPR peptide stretches strongly bind to the anti-SIRPα antibody and thus represent the core epitope (Fig 1). Linear peptides or simple looped peptides do not bind to anti-SIRPα antibody (data not shown). To follow up, a comprehensive replacement analysis of the whole combinatorial mimic revealed residues crucial and important for the antibody binding. In such replacement analyses every residue is substituted by all canonical amino acids (Fig 2).

Conclusion:

Pepscan's exclusive expertise in CLIPS chemistry and strategies for epitope mapping allow addressing challenging discontinuous epitopes with single residue precision.

Discontinuous epitope mapping: "by domain" combinatorial CLIPS library

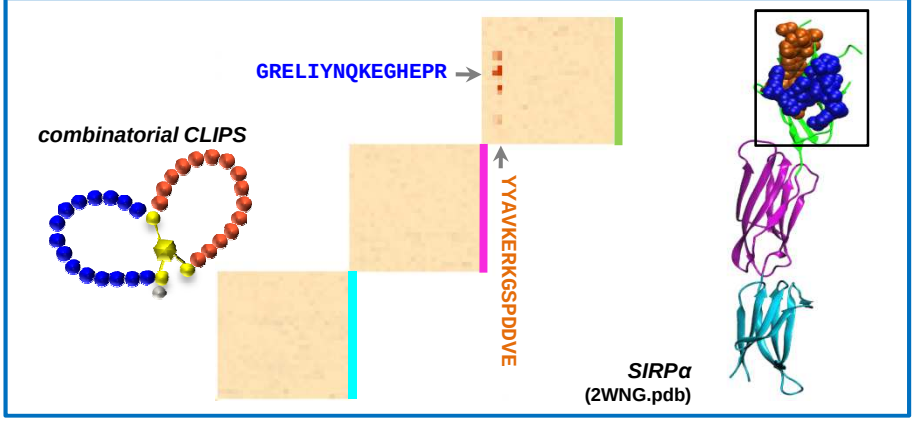

Figure 1: *Identification of the discontinuous epitope for anti-SIRPα using looped matrix CLIPS peptides derived from the sequences of each individual domain of human SIRPα. Individual domains are colored in green, magenta and cyan as well as corresponding heatmaps that result from matrix analyses. Only mimics containing residues YYAVKERKGSPDDVE and GRELIYNQKEGHEPR located within the first Ig-domain (green) were bound. On the right is the mapping of the identified stretches onto 3D structure of SIRPα.*

Core epitope: substitution pinpoints individual residue contributions

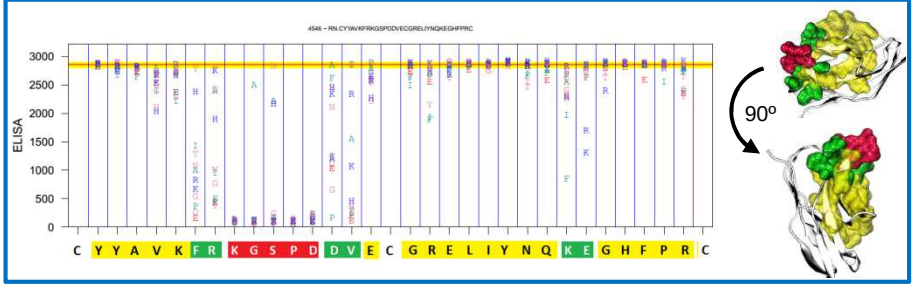

Figure 2: *Single residue precision is obtained by substituting the residues within the context of the epitope mimic. Most mutations do not affect binding (in yellow); however residues KGSPD in red cannot be replaced and substitutions of residues FR, DV and KE in green affect binding to a lesser degree. Cysteine residues were not replaced to maintain the mimic's structure. The outcome of the replacement analysis is similarly visualized in 3D on the right.*

The benefits of CLIPS Precision Epitope Mapping

- Suitable for all types of epitopes
- Discontinuous, conformational, and linear
- Applicable to all kinds of challenging 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	Four 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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