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Rapid B Cell Linear Epitope Mapping

ProImmune's Rapid B Cell Linear Epitope Mapping service provides mapping of the B cell linear epitopes of a protein or polypeptide sequence using human sera or plasma samples submitted by the client.

There are two main classifications of B cell epitopes:

- Linear, or continuous, epitopes are defined by the primary amino acid sequence of a particular region of a protein. The sequences that interact with the antibody are situated next to each other sequentially on the protein.
- Epitopes that are defined by the conformational structure of the native protein. These epitopes may be continuous or discontinuous, i.e. components of the epitope can be situated on disparate parts of the protein, which are brought close to each other in the folded native protein structure.

Conformational epitopes are thought to form the majority of strong antibody binding epitopes on most proteins. However numerous conformational epitopes may also be recognizable as linear epitopes, although with lower affinity. Linear B cell epitopes typically vary from 5 to 20 amino acids in length. If the antibody-binding region of the protein is unknown, an overlapping peptide library can be generated using a specific peptide length and offset, for example, 15mers offset by 5 amino acids (i.e. overlapping by 10).

Mapping linear epitopes in a protein has the following benefits:

- It can be done quickly with well-defined, easily controllable high throughput technologies, which include standard rapid synthesis of overlapping peptide libraries and standard immunoassays.
- Although not all conformational epitopes may be mapped, linear B cell epitope mapping can be a quick way of getting a relative ranking of antigenic regions within proteins and between different proteins in a group.

Purpose

Using our epitope mapping service, the specific linear protein segments from any larger protein or peptide, that bind to antibodies in a given sample such as sera or plasma, can be identified. In this way, 'hot-spot' areas of the protein can be discovered. Alternatively, if these areas of the protein sequence are already defined, the optimal epitope sequence can be elucidated using a peptide library with an offset of 1, or with a truncated peptide library, or with a series of peptides with single amino acid substitutions.

Applications

Definition and ranking of individual linear epitopes in an antigen

Although protein antigens may contain both linear and discontinuous epitopes, detailed knowledge of the linear epitopes alone may be a useful means of comparing the antigenicity of candidate proteins.

Identification of peptides for immunization

If a strong candidate linear epitope can be found in a protein antigen, this short peptide sequence can be used to produce polyclonal or monoclonal antibodies against the target protein, with the advantage that the binding sequence in the protein will already be known.

Discovery of cross-reactive determinants between related proteins

Determining a linear epitope that occurs on several related proteins gives useful information about the potential cross-reactivity of immune responses between these proteins.

Definition of strongly antigenic regions in proteins

Clusters of linear epitopes may indicate regions of elevated antigenicity and activity in a protein and may be useful in identifying protein sub-units relevant to the research application. For example, a sub-unit of a protein may confer most of the antigenicity of the whole protein and be easier to manufacture than the whole protein. In addition, to reduce the risk of unwanted side effects in vaccination it may be of benefit to use a sub-unit of a protein that has fewer epitopes that are cross-reactive with self-proteins.

Comparison of different serovars or subtypes in terms of their relevant antigenicity

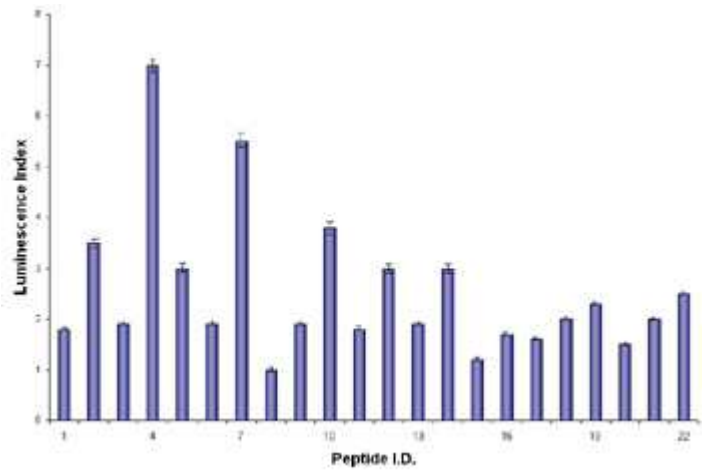
Comparative results from linear mapping using peptides with sequence variation can shed light on the antigenicity of proteins from different strains of an organism. This application may also show whether vaccination produces an immune response that is relevant across a number of serovars or subtypes.

Combining linear epitope mapping with epitope prediction

Comprehensive B cell epitope prediction and linear epitope mapping can be carried out when both the 3D structure and the protein sequence are known. However, the 3D structure is not available for many proteins, especially transmembrane proteins, which are particularly difficult to crystallize. In vaccine development, transmembrane proteins are of special interest when they have a domain on the surface of the pathogen that could be bound by antibodies. Linear epitope mapping alone, or combined with epitope prediction, can give an insight into the antigenicity of transmembrane proteins.

The B Cell Linear Epitope Mapping Service In Detail

Our B Cell Epitope Mapping Service uses standard immunoassay methodologies to screen plasma or sera samples submitted by the customer. Based on the proteins of interest, ProImmune can design and synthesize an appropriate peptide library for the project. Our services include cost-effective solutions for a wide range of project requirements: e.g. from a handful of peptides screened against a similar number of antibody samples, to hundreds or even thousands of peptides screened against hundreds of antisera using ProArray™ Custom Peptide Microarrays.



Biotinylated custom peptides are bound to a microtiter plate and plasma/sera samples are added at dilutions of 1:200 and 1:400, along with controls to determine background signal. Peptides that are bound by antibodies in the samples are detected using an HRP-conjugated polyclonal antibody, and visualized by chemilumin-escence. Results are shown for linear epitope mapping of 22 peptides with one plasma sample.

The customer receives a report outlining the methods used and all results from the assay, proposing, ranking and discussing any predicted epitopes. Data generated from the assay will depend upon the quality of the antibody being tested. It should be noted that sera and plasma samples sometimes contain multiple antibodies, which may give weak signals and/or bind to several different peptides. The data generated from such samples can still provide good information about which regions of a protein sequence are most antigenic, and highlight areas on which to carry out further investigation.

Discontinuous epitopes that depend on the correct 3D conformation of the protein, may not be detected by linear epitope mapping, or may only give a weak signal. If the 3D structure of the protein is known, it may be possible to synthesize reconstructed discontinuous epitopes as linear peptides to be included in the assay.

Case Study: Defining autoantibody binding to citrullinated target proteins in Rheumatoid Arthritis using ProArray™ Peptide Arrays

Professor Lars Klareskog and his team at the Karolinska Institute in Stockholm, Sweden, have a long-standing research interest in Rheumatoid Arthritis (RA)[1]. RA onset is associated with MHC class II–dependent activation of adaptive immunity. A variety of proteins (such as vimentin, α-enolase, type II collagen, and fibrinogen) undergo posttranslational modification of arginine to citrulline over time, and a working hypothesis is that this may be aggravated by smoking or bacterial infection. Autoantibodies towards these modified proteins may complex with their target citrullinated proteins as part of a multistep process for RA development.

Increasing the number of B cell epitopes on candidate autoantigens is central when dissecting this complex disease and with that intent researcher Lena Israelsson turned to ProImmune's B cell Epitope Mapping Service.

ProImmune synthesized a series of novel and previously identified peptides from RA-associated proteins in both their native and their citrullinated variants, then spotted them onto multiple ProArray™ slides for screening. Sera collected from RA patients were assayed for binding to the paired native and citrullinated peptides. Across the set of patient samples, differences were apparent between citrullinated peptides and their unmodified counterparts, as shown in figure 1, below.

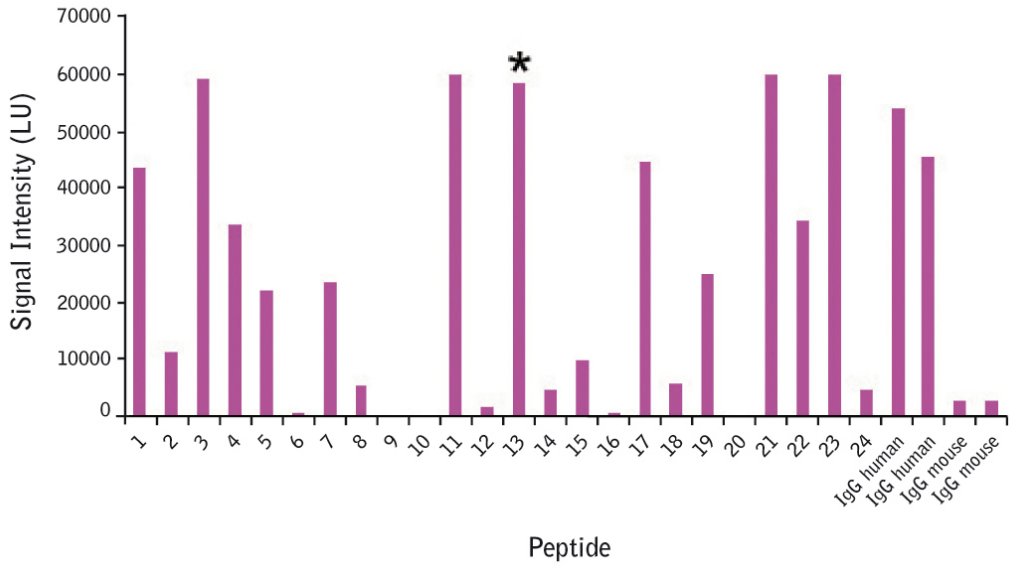


Figure 1: ProArray™ Results from a test serum sample for binding to 12 peptide pairs (native and citrullinated) from RA-associated proteins. Asterisk indicates result from one citrullinated fibrinogen peptide sequence (peptide 13), shown next to result from the native sequence (peptide 14).

For each of the peptide pairs screened, it was evident that the patient antibodies bound more strongly to the citrullinated variant than the native peptide. ProImmune's peptide array based mapping is sufficiently sensitive to highlight these differences in binding. While further validation work is required, provisionally, these results support the hypothesis that citrullinated epitopes could be important for the development of RA. The data generated by ProImmune in a short space of time, helped shortlist some of the novel peptides for further studies at the Karolinska Institute.

[1] Immunity to Citrullinated Proteins in Rheumatoid Arthritis
Klareskog, L., *et al* Annual Review of Immunology (2008) Vol. 26: 651-675 PMID: 18173373