Antibody-Antigen Binding: Shape Matters

Functional protein microarrays are powerful tools for immunoprofiling. The information encoded by the distinct repertoire of antibodies within an individual provides important insight into the disease state. However, capturing this information requires a well-designed protein microarray.

Functional protein microarrays emerged in the early 2000's, a few years behind DNA microarrays. Both technologies were designed for high throughput analysis and utilized different detection techniques. DNA microarrays are miniaturized hybridization assays and functional protein microarrays are miniaturized indirect immunofluorescent assays. In a functional protein microarray, proteins are printed onto a planar surface. The test sample is most often serum, replete with host antibodies. Serum antibodies bind specifically to complimentary protein. A fluorescently tagged secondary antibody, directed against the species of the serum antibodies, is applied to illuminate the protein-serum antibody complex. Functional protein microarrays can contain thousands of proteins, fragments, or peptides, enabling immunoprofiling of individuals and populations, identifying unique immune experiences, including early disease. Functional protein microarrays are highly dependent on antigen-antibody binding specificity.

In biological systems, proteins require a specific, three-dimensional configuration to perform their functions. During translation, the ribosome creates a linear, continuous chain of amino acids. This amino acid chain folds into a secondary structure driven by hydrogen bonding between amino acids in close proximity. The protein continues to fold into its tertiary structure driven by forces such as hydrogen bonds, van der Waals forces and hydrophobic interactions, consequently juxtaposing distant amino acids and different atomic groups that make up the peptides.

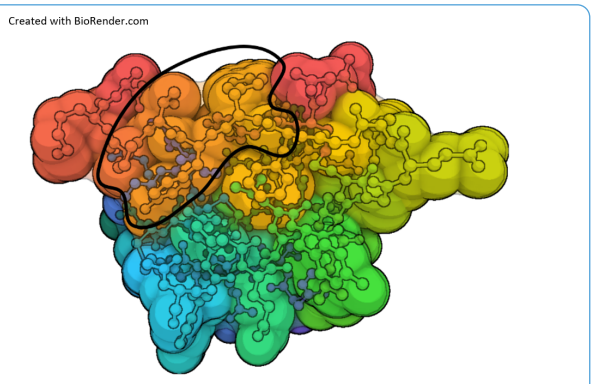

Figure 1. Antibody epitopes form from different atomic groups from discontinuous amino acids that form a surface (outline) when the protein folds. A contact map of insulin is shown with atomic groups overlayed. Blue, orange, and dark orange atomic groups fold over each other to form the epitope.

Each atomic group represents a different section of the protein, and when the protein folds, these groups arrange near each other to form the epitope, a unique surface recognized by a specific antibody (Figure 1). Because, in most cases, the amino acids in the tertiary structure are not continuous, the resulting antibody binding site is called a discontinuous epitope (Figure 2) (Barlow et al., 1986; Van Regenmortel, 1996).

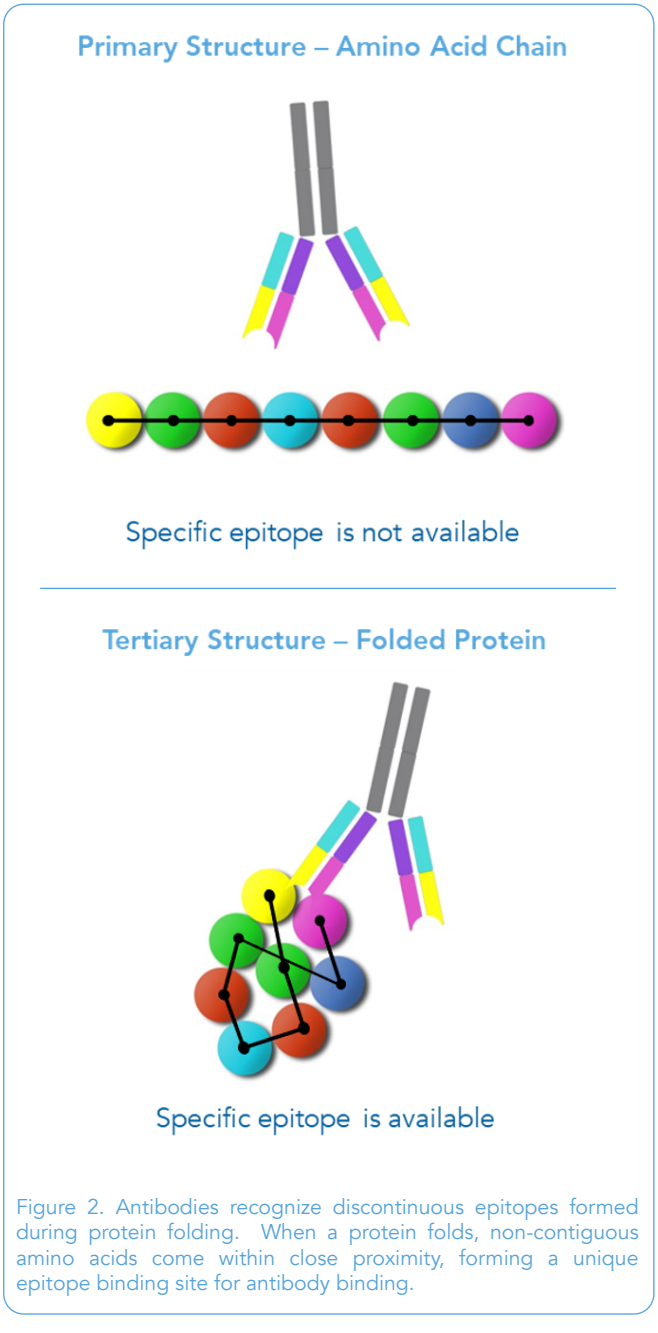

Figure 2. Antibodies recognize discontinuous epitopes formed during protein folding. When a protein folds, non-contiguous amino acids come within close proximity, forming a unique epitope binding site for antibody binding.

The atom groups make up the epitope, not the amino acid sequence. X-ray crystallography has been used to visualize the epitope with the amino acid sequence mapped to illustrate the discontinuity. If the protein unfolds, is denatured or fragmented, then the epitope is lost (Van Regenmortel, 2006, 2016). There are multiple factors that can affect protein folding including temperature, pH, other molecules, and the underlying amino acid sequence. Intracellular chaperones and folding enzymes ensure the protein is correctly folded into its functional three-dimensional structure. In some cases, proteins are made up of subunits and those subunits must also come together to be functional.

Ninety percent of antibodies recognize the discontinuous epitopes formed by protein folding (Van Regenmortel, 1996). This becomes a challenge for manufacturing protein microarrays because high quality data relies on retaining these epitopes through production and immobilization of properly folded proteins, a procedure difficult to control in a manufacturing setting. When the epitope is lost, the antibody recognition site disappears, non-specific binding increases, and the rate of false positives and false negatives also increases. Sengenics KREX protein folding technology is designed to ensure the protein array consists of full length, properly folded proteins. In this patented technology, a biotin carboxyl carrier protein (BCCP) is coded in-frame with each array protein as a folding marker. A misfolded or fragmented protein results in BCCP misfolding, masking its biotinylation site and preventing it from binding to the streptavidin coated array surface (Figure 3). This technology maintains discontinuous epitopes and ensures optimal antibody-epitope binding.

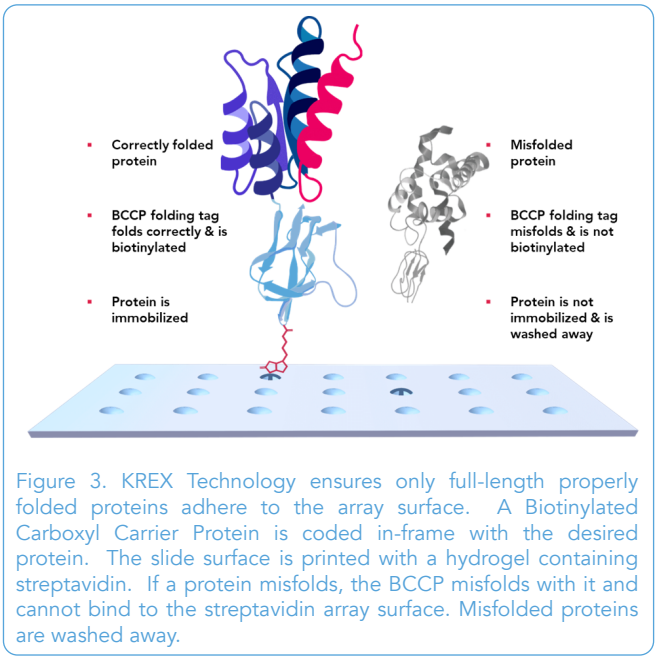

Figure 3. KREX Technology ensures only full-length properly folded proteins adhere to the array surface. A Biotinylated Carboxyl Carrier Protein is coded in-frame with the desired protein. The slide surface is printed with a hydrogel containing streptavidin. If a protein misfolds, the BCCP misfolds with it and cannot bind to the streptavidin array surface. Misfolded proteins are washed away.

Antibodies circulate in the blood and lymph carrying the disease history of an individual. Techniques such as protein microarrays can read this history using the well-established technique of indirect immunofluorescence. Using technology that addresses the nature of antibody-antigen binding ensures relevant results with high signal to noise.

References

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