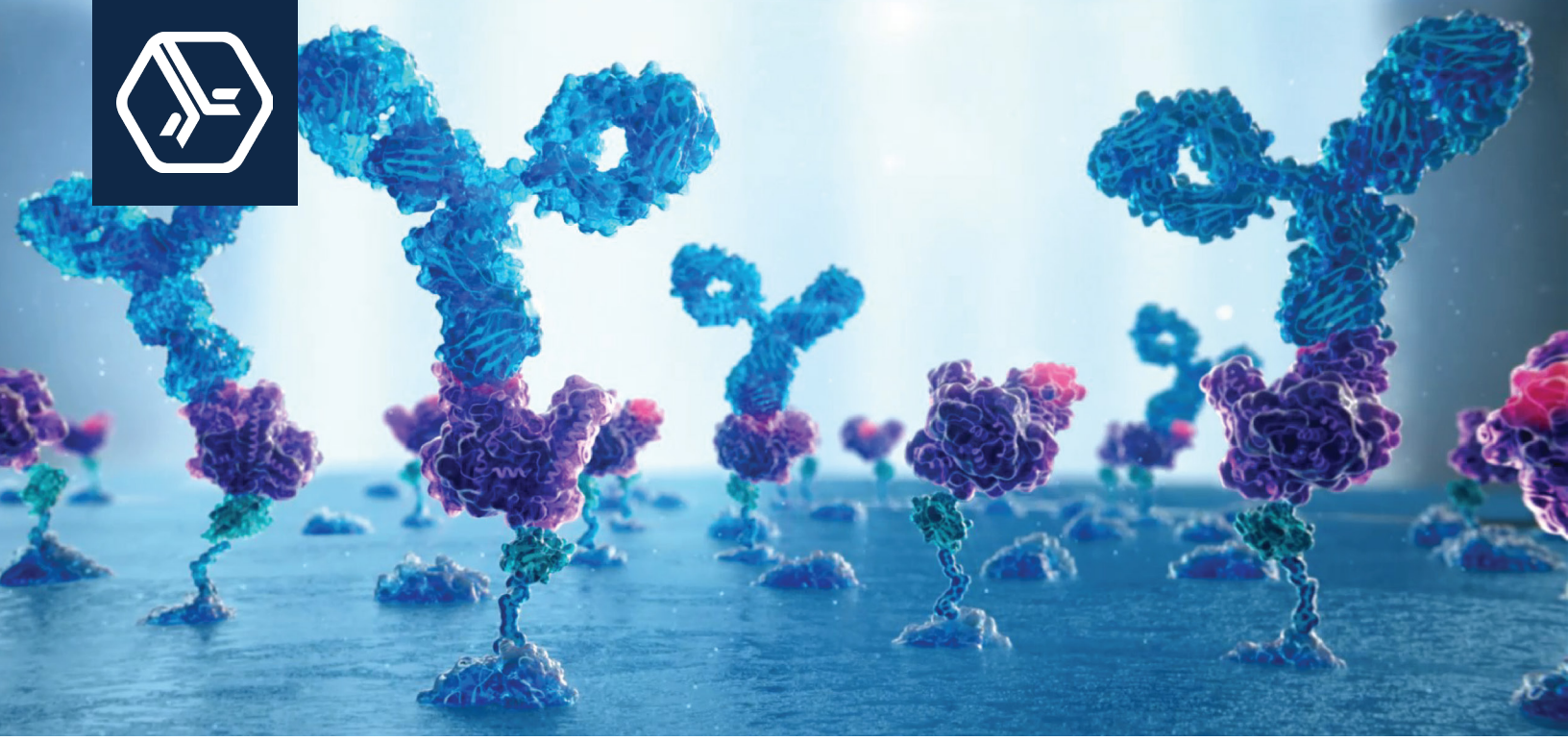

WHITE PAPER
ANTIBODY-ANTIGEN BINDING

Antibody-Antigen Binding: Shape Matters

- Understand the mechanism of protein microarrays for detailed immunoprofiling and disease marker identification
- Learn about the critical role of protein structure in antibody recognition
- Explore advanced KREX® technology that ensures precise antibody binding to correctly folded proteins

sengenics

Introduction

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, effectively capturing this information requires a well-designed protein microarray.

Antibody Profiling with Protein Microarrays

Protein microarrays, which surfaced in the early 2000s, have become pivotal tools for high-throughput analysis in proteomics. As miniaturized indirect immunofluorescent assays, protein arrays display a vast array of antigens (proteins, peptides, or fragments) on a solid surface, typically a glass slide, in an addressable format.

A test sample — often serum rich in host antibodies — is then applied to the array (Figure 1). The antibodies target and bind specific antigens on the microarray.

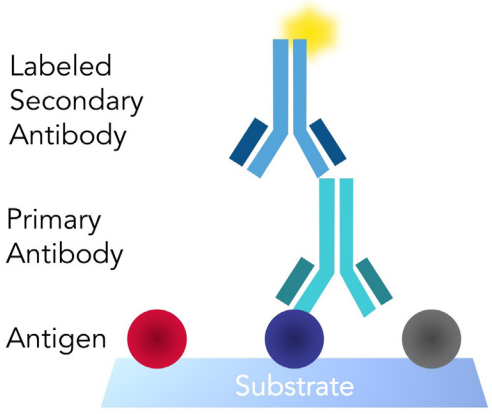

Figure 1. Schematic of an antibody binding to an antigen on a protein microarray

Following antibody binding, a fluorescently-tagged secondary antibody specific to the host species of the sample is added. This secondary antibody binds to the primary antibodies attached to the proteins, allowing for the indirect visualization and detailed immunoprofiling of antibody-antigen complexes.

The fluorescence intensity is proportional to the quantity of antibodies, whereas the fluorescence location on the array facilitates the identification of the targeted antigen.

Depending on the array, multiple antibody isotypes (i.e., IgG, IgM, IgA, IgE) with unique effector functions may be analyzed simultaneously. This capability is made possible with the use of secondary antibodies that are labeled with different fluorophores. Measuring various isotypes offers a more comprehensive view of disease, delivering valuable insights into the timing and localization of the immune response (1).

Protein arrays are instrumental in studying immune responses across both individual and population levels, revealing unique immune signatures and potentially identifying markers of early disease states. The effectiveness of functional protein microarrays heavily relies on the specificity of antibody-antigen interactions, which determines the accuracy of the data.

Overall, functional protein microarrays offer a powerful platform for exploring the complex dynamics of the immune system, highlighting their critical role in advancing biomedical research and diagnostic capabilities.

Protein Folding and Epitope Formation

Proteins must adopt precise three-dimensional shapes to carry out their diverse biological functions effectively. This process begins during translation, where the ribosome synthesizes a linear chain of amino acids. As this chain emerges from the ribosome, it starts to fold into a complex structure.

Initially, the amino acid chain forms what is known as the secondary structure, primarily through hydrogen bonds between amino acids that are near each other. This structure then progresses into a more complex tertiary structure, orchestrated by a variety of forces including hydrogen bonds, van der Waals forces, and hydrophobic interactions. These forces help to bring together distant amino acids and various atomic groups, which are part of the peptide chain, into close proximity.

As the folding process completes, specific regions of the protein — the atomic groups — come together to form what is known as an epitope. This epitope constitutes a unique molecular landscape on the protein surface, specifically recognized by antibodies. Interestingly, the amino acids that make up this epitope are often not sequential along the peptide chain but are brought together as the protein folds into its three-dimensional shape. This type of epitope, formed by the juxtaposition of non-continuous amino acid sequences, is referred to as a conformational or discontinuous epitope (Figure 2) (2,3).

Protein folding, which determines the formation and stability of epitopes, is influenced by several factors including temperature, pH, interactions with other molecules, and the primary amino acid sequence itself. Inside cells, chaperones and folding enzymes play vital roles in ensuring proteins achieve and maintain their functional three-dimensional structures. Additionally, some proteins are composed of multiple subunits that need to assemble correctly to become functional.

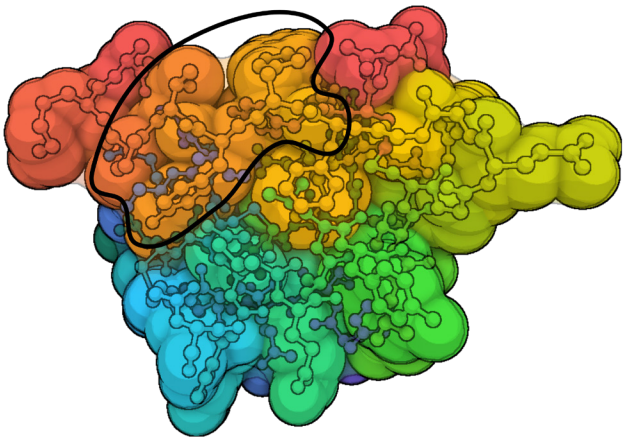

Figure 2. Illustration of the three-dimensional structure of the protein, insulin. The black outline indicates a conformational epitope comprised of discontinuous amino acids.

The Vital Role of Protein Shape in Antibody Recognition

The formation of conformation epitopes is a fascinating aspect of protein biochemistry, crucial for the immune system's ability to recognize and respond to complex pathogens. Techniques like X-ray crystallography have been instrumental in mapping discontinuous epitopes, revealing how non-sequential amino acids come together to form a functional binding site. However, if a protein unfolds, is denatured, or fragmented, the structured arrangement of these atomic groups collapses, resulting in the loss of the epitope (4,5).

Remarkably, it is estimated that **90% of humoral antibodies recognize these complex conformational epitopes** (3), highlighting their importance in immune response. It also underscores the sophistication of molecular interactions in biological systems and their relevance in antibody binding and immune specificity (4,5).

Scan this QR code to watch a quick, 1-minute video on how protein folding plays a crucial role in immunoprofiling

Ensuring Accuracy with Protein Microarrays

Peptides or proteins that are misfolded or fragmented on protein microarrays can lead to non-specific binding and higher rates of false positives and negatives. For example, misfolded or denatured proteins may expose or generate new epitopes that would otherwise be occluded or not present *in vivo*. Consequently, the accuracy of antibody-epitope binding — and by extension, the quality of data derived from these microarrays — depends crucially on maintaining the proteins in their correct, folded state.

To preserve the critical conformational epitopes recognized by humoral antibodies, Sengenics has developed KREX® protein folding technology to generate functional protein arrays with full-length and correctly folded proteins (Figure 3). This is made possible through a small 10 kDa subunit of the biotin carboxyl carrier protein (BCCP), which is incorporated into each protein on the array. BCCP acts as a folding marker; if a protein is misfolded or fragmented, the BCCP also misfolds, hiding its biotinylation site and preventing attachment to the streptavidin-coated array surface (6,7). This is a unique feature of BCCP compared to other tags for immobilization like glutathione S-transferase (GST) and maltose-binding protein (MBP).

Importantly, the surface of Sengenics protein arrays mimics an aqueous environment (6). This allows the proteins to behave as if they are freely suspended in solution, contributing to their natural folding and function.

Finally, human proteins on Sengenics arrays are expressed in insect cells, which more closely replicate protein processing of mammals compared to bacteria or yeast (6,7). For instance, protein expression in the commonly employed bacterial system, *Escherichia coli*, frequently results in insoluble, poorly folded proteins. Yeast expression systems, such as *Saccharomyces cerevisiae* and *Pichia pastoris*, do not mimic glycosylation patterns of mammalian cells, and the harsh cell lysis conditions often lead to denatured or fragmented proteins. Therefore, the use of insect cells aligns more closely with mammalian systems, significantly improving the functional expression of human proteins.

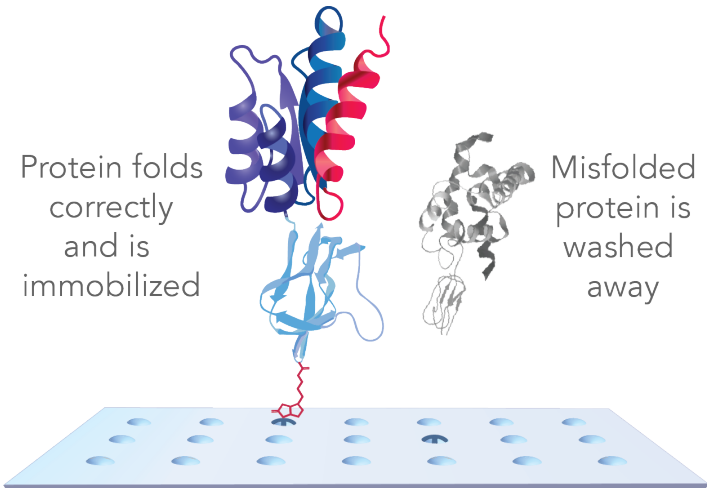

Figure 3. KREX technology for precise antibody profiling

Concluding Remarks

Leveraging technology that meticulously addresses the nature of antibody-antigen interactions ensures that protein microarrays deliver results with high relevance and low background noise. By focusing on maintaining the integrity of protein structures and their correct folding, Sengenics arrays provide precise, reliable data essential for advancing our understanding of immune responses and improving diagnostic accuracy. This approach is crucial for minimizing false readings and enhancing the overall effectiveness of immunoprofiling tools in medical research and clinical diagnostics.

Contact us

Sengenics develops key solutions for biological research, specializing in the discovery and validation of autoantibody biomarker signatures. For more information on how Sengenics can advance your research, email us at enquiries@sengenics.com or visit our website at sengenics.com.

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