Exploring Diverse Protein Interactions using the Sengenics Protein Array

Technology background

Recombinant protein expression is a fundamental technique that underpins clinical diagnostics, drug discovery and screening, vaccine development and pure research for elucidating mechanisms of disease development and progression. However, high-throughput production of correctly folded and functional, full-length human proteins has a very high failure rate. Protein folding is a highly complex process requiring a combination of an aqueous environment, chaperones, post-translational modifications and the formation of multimeric structures held together by covalent bonds. Any deviation from the correct sequence of events can result in a misfolded protein. Loss of protein function is directly linked to misfolding. Use of misfolded proteins in downstream assays and interactions studies can result in identification of false positive biomarkers.

The Sengenics KREX™ technology utilises the biotin carboxyl carrier protein (BCCP) as a folding marker and solubility enhancer which results in high-throughput expression of full-length, correctly folded and functional proteins. BCCP-protein fusions are capable of being biotinylated either *in vivo* or *in vitro*, allowing the use of highly specific biotin-streptavidin interaction for surface capture. As biotinylated proteins bound to a streptavidin-coated surface show negligible dissociation, this interaction therefore provides a vastly superior means for tethering proteins to a planar surface and is ideal for applications such as protein microarrays, glass micro-titer plates, SPR and bead-based assays.

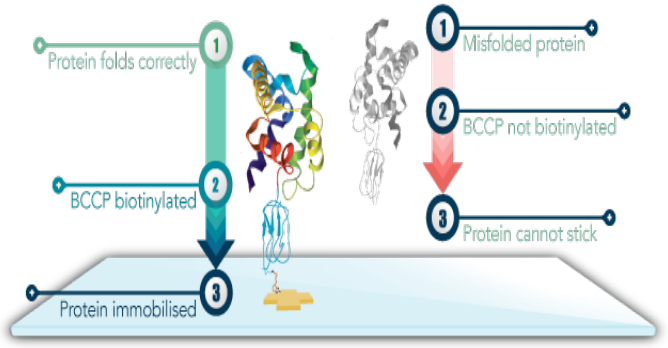

Figure 1. The BCCP folding marker acts as a marker for correctly folded proteins. Proteins will be immobilised on the array only when they are properly folded and biotinylated on the BCCP folding marker.

Introduction

Leveraging the KREX™ technology, we have developed a fully quantitative protein microarray platform which affords the simultaneous screening of over thousands of functional proteins for various medical and therapeutic proteomics applications. All arrayed proteins are assayed simultaneously under identical conditions resulting in quantitative and genuinely comparative data. It is a highly reproducible, miniaturised assay platform for systematic, high-throughput studies of protein function.

This platform allows us to elucidate the roles of various different proteins in the context of its interaction with other proteins, peptides, DNA, RNA and small molecules. Utilising the KREX™ technology ensures that all immobilised proteins to any purpose-designed solid support platforms (e.g. protein microarrays) are full-length, correctly folded and functional, with both linear and conformational epitopes captured, thus ensuring any interactions that take place are true interactions, and not due to non-specific attachment or binding of proteins which usually occurs with other high-throughput proteomics platforms.

TECHNICAL PERFORMANCE

- Expression of correctly folded and functional proteins with a 98% success rate.
- Fully quantitative clinical-grade assay metrics. Dynamic range is linear up to five orders of magnitude.
- Excellent reproducibility and precision with a mean CV% below 4% between replica arrays.
- Highly sensitive with a limit of detection of approximately 1:1,000,000 serum dilution and autoantibody titer of 190 pg/mL.
- Exceptional specificity and accuracy: non-specific binding eliminated as all proteins are immobilised as functional and correctly folded BCCP-fusions.

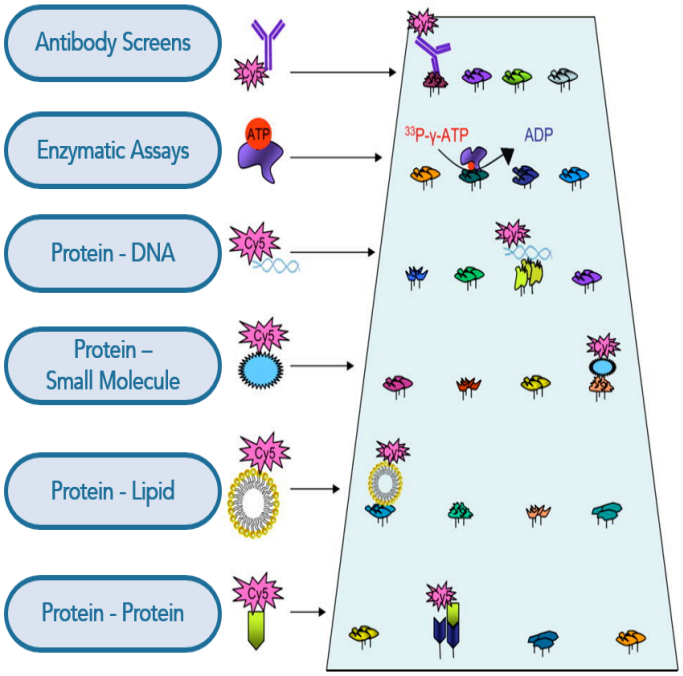

Figure 2. Protein interaction with different types of analytes. Hall, Ptacek & Snyder, 2007.

Case studies

1. **NYO-ESO-1 vaccine interaction in Melanoma patients** *(Beeton-Kempen N. et. al., 2014)*

Study Design:

- 46 melanoma patients, vaccinated with NY-ESO-1 repeatedly (D1, D28, D56, D140, D231).
- Inclusion criteria: Tumor expression of NY-ESO-1 antigen by immunohistochemistry.
- Serum samples taken at D0 and at 2 weeks after each vaccination event.
- 127 sera analysed on the Sengenics protein array platform covering a total of 123 tumor associated antigens (TAAs).

Results

As regulatory T cells can interfere with immune responses in patients with cancer, using a small dose of cyclophosphamide to suppress regulatory T cells increased patient responses to the NY-ESO-1 ISCOMATRIX® vaccine. This also increases the autoantibody titer,

indicating that autoantibodies can be used for monitoring treatment response in cancers.

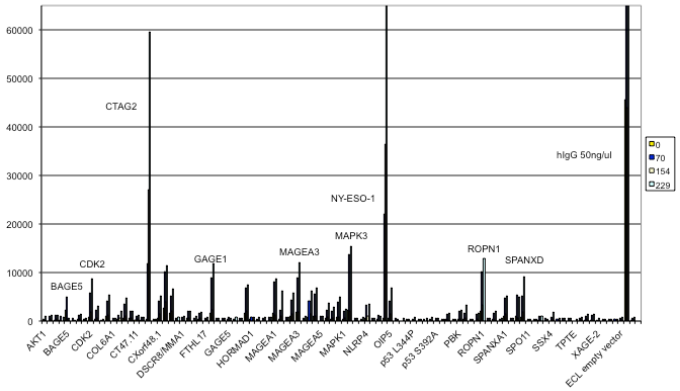

Figure 3. Complete data for individual patients: In a few patients, many autoantibodies apparently increased in titer post-vaccination.

2. **DNA-binding analysis using a p53 protein microarray** *(Boutell, J.M. et. al., 2004)*

Study Design:

Protein-protein interactions between p53 variants and, i) a regulatory onco-protein, ii) a regulatory kinase resulting in on-chip phosphorylation

Mutation	DNA binding		MDM2	CKII
	B_{max}/% wild-type	K_d/nM		
Wild-type	100 (90–110)	7 (5–10)	+	+
W23A	131 (119–144)	7 (5–10)	–	+
W23G	84 (74–94)	5 (3–9)	–	+
R72P	121 (110–132)	9 (7–13)	+	+
P82L	70 (63–77)	7 (5–10)	+	+
M133T	ND		+	+
Q136X	No binding		+	–
C141Y	ND		+	+
P151S	ND		+	+

Figure 4. The binding affinity constant (K_d) and B_{max} value for some of the variants.

Results

Proteins with mutations outside of the DNA-binding domain generally had near wild-type affinity for DNA. By contrast, total loss of binding was observed for mutations resulting in truncated proteins and mutations that monomerise the protein.

Custom protein array development

Viral infections are mediated by several protein–protein interactions. Protein domains are basic units defining protein interactions and mutations at protein interfaces can reduce or increase their binding affinities by changing protein electrostatics and structural properties. During the course of a viral infection, both pathogen and cellular proteins are constantly competing for binding partners. Endogenous interfaces mediating intraspecific interactions—viral–viral or host–host interactions—are constantly targeted and inhibited by exogenous interfaces mediating viral–host interactions. Blocking such interactions is the main mechanism underlying antiviral therapies (Brito and Pinney, 2017). A protein array consisting of antigens from different bacterial/viral strains can be developed and used to evaluate human sera. The array will allow simultaneous examination of the magnitude of antibody responses, the isotype of such antibodies, and the breadth of the bacterial/viral strain recognition.

Current Study:

Protein X is a membrane protein (MP) of virus Z, key to the pathogenesis of infection, it is therefore important as a potential vaccine candidate. The aim is to develop Protein X-based vaccine that can elicit serum antibodies capable of suppressing virulence.

Strategy:

Detect and evaluate Z membrane protein, protein X, as a vaccine component. Detect the MPs in which antibody response occur predominantly, and evaluate the MPs detected for their potential as vaccines.

Expected Results:

High efficacy vaccine candidate for virus Z infection.

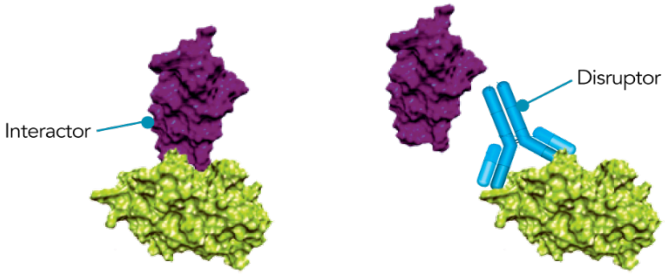

Figure 4. A good vaccine candidate should be able to disrupt, block or inhibit an interaction that is involved in viral entry or attachment.

References

1. Beeton-Kempen N, Duarte J, Shoko A, Serufuri JM, John T, Cebon J, Blackburn J. Development of a novel, quantitative protein microarray platform for the multiplexed serological analysis of autoantibodies to cancer-testis antigens. International journal of cancer. 2014;135:1842–1851.
2. Boutell, J.M., Darren, J.H., Godber, B.L., Kozlowski, R.Z. & Blackburn, J.B. (2004) Functional protein microarrays for parallel characterisation of p53 mutants. *Proteomics* 4, 1950-1958.
3. Brito AF and Pinney JW. 2017. Protein–Protein Interactions in Virus–Host Systems. Front. Microbiol. https://doi.org/10.3389/fmicb.2017.01557.
4. Hall DA, Ptacek J and Snyder M. 2007. Protein microarray technology. Mech Ageing Dev.; 128(1): 161–167.

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