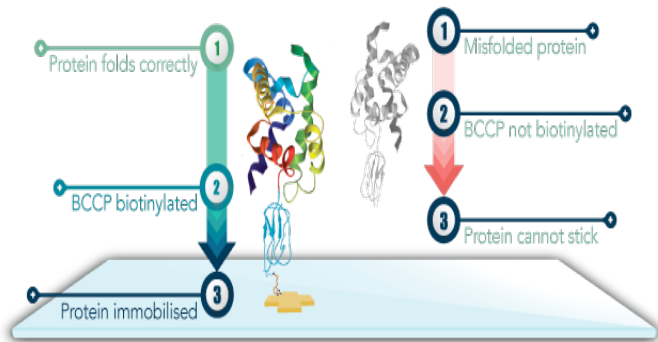


# Autoantibody Discovery using a Novel Microarray of Functional Proteins

## 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

In addition to producing antibodies against foreign molecules, the immune system generates antibodies to self-antigens (“autoantibodies”) in response to many pathological processes. Autoantibodies have several properties which make them excellent indicators of disease and their detection forms the basis of many *in vitro* diagnostic tests. It is believed that autoantibodies are generated through over-expression, mutation, release of proteins from damaged tissues, mis-folding or mis-presentation of proteins which leads to their recognition by the immune system.

Unlike other serological targets, autoantibodies are stable, highly specific, easily purified from serum, and are readily detectable with well-validated secondary reagents. Due to their inherent amplification within the immune system, autoantibodies are relatively abundant and are easily measured, making them ideal for early diagnosis of disease.

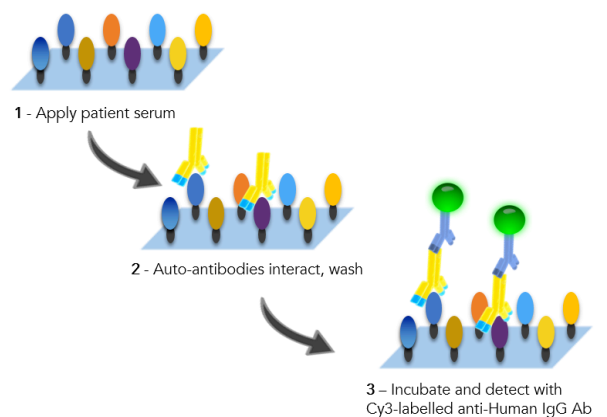
Leveraging the KREX™ technology, we have developed a fully quantitative protein microarray platform which affords the simultaneous screening of over thousands of functional proteins for various medical and therapeutic proteomics applications. All arrayed proteins are assayed simultaneously under identical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

### TECHNICAL PERFORMANCE

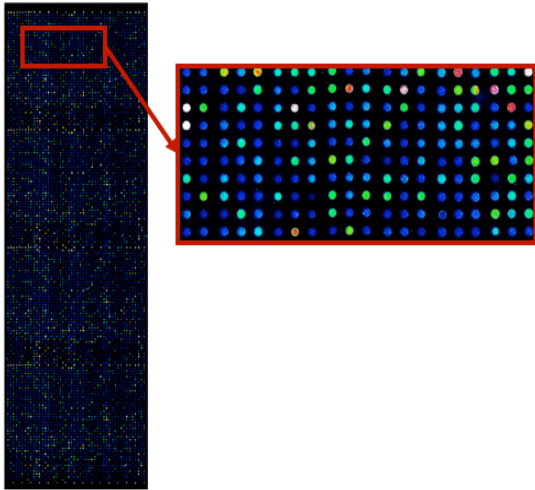
- Expression of correctly folded and functional proteins with a 98% success rate.
- Fully quantitative clinical-grade assay metrics. Dynamic range is linear up to five orders of magnitude.
- Excellent reproducibility and precision with a mean CV% below 4% between replica spots.
- Highly sensitive with 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.

TYPES OF DISEASES	BIOMARKERS IDENTIFIED
Cancer	Protective
Autoimmune Diseases	Diagnostic
Neurodegenerative Diseases	Screening
Infectious Diseases	Therapeutics

This platform has been successfully used to identify predictive, pathologic and protective biomarkers for cancers, autoimmune, neurodegenerative and infectious diseases. The autoantibody assay protocol is summarised in Figure 2. For each protein, interactions are measured in the form of relative fluorescence units (RFU) using any open format Microarray Scanner at 10µm resolution. Each image is then saved as a 16-bit TIFF file (Figure 3).



**Figure 2.** Summary of the autoantibody biomarker discovery protocol.



**Figure 3.** An example tiff. image of the Sengenics 1600+ Immunome™ protein array.

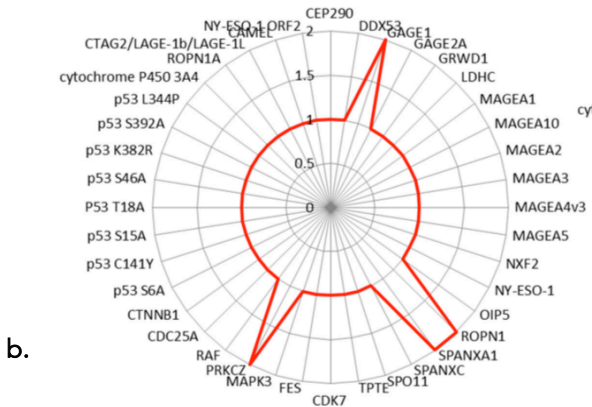
**Case studies**

**1. Autoantibody Biomarker Discovery for Prostate Cancer using our miniature protein microarray platform; CT100+ array. (Adeola, H, et. al., 2016)**

This study aimed to elucidate the role of 123 tumour associated antigens (TAAs) using the Sengenics microarray platform in blood samples from 20 PCa, 32 benign prostatic hyperplasia (BPH) and 15 disease control (DC) cohorts.

**Results**

Linear quantitation showed four antigens, GAGE1, ROPN1, SPANXA1 and PRKCZ having higher autoantibody titres in PCa serum as compared to BPH where MAGEB1 and PRKCZ were highly expressed.



**Figure 3.** Radar plot showing GAGE1, ROPN1A, SPANXA1 and PRKCZ was highly expressed in PCa.

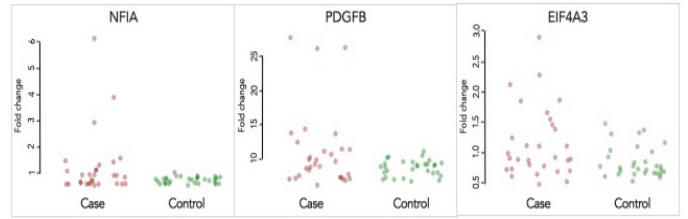
**2. Study of Neurodegenerative Diseases using the Sengenics 1600+ Immunome Protein Microarray (Suwarnalata, G, et. al., 2016)**

Helicobacter pylori (H.pylori) positivity has been associated with greater motor severity in Parkinson’s disease (PD). To investigate this association, 30 H. pylori-seropositive PD samples were designated as case and 30 age- and gender-matched H. pylori-seronegative PD samples were used as controls.

**Results**

This study identified 13 significant autoantibodies based on ranking using the Sengenics penetrance fold change analysis method. Amongst these elevated autoantibodies in H. pylori-seropositive PD,

NFIA, PDGFB and eIF4A3 have previously been identified as essential proteins involved in neurological function.



**Figure 4.** Fold changes between H. pylori-seropositive PD samples (Case) and H. pylori-seronegative (Control) groups for autoantibodies showing higher activity in the case group.

**3. Autoantibody profiling in Infectious Diseases using the Sengenics 1600+ Immunome Protein Microarray (Liew, J., et. al., 2015)**

A total number of 22 serum and plasma samples were collected from 11 patients with PCR and microscopically confirmed plasmodium knowlesi malaria infection and 11 age- and gender-matched, healthy individuals (n = 11) who are negative for malaria infection. A comparison analysis of data generated from the protein microarray experiment between these 2 cohorts was performed for the identification of potential autoantibody biomarkers.

**Results**

This study identified 24 antigens with high reactivity with serum antibodies which are involved in inflammatory processes in the host. These antigens could serve as potential biomarkers for cases of asymptomatic malaria and mild malaria or predictive markers for severe malaria.

**References**

- Adeola, H. A., Smith, M., Kaestner, L., Blackburn, J. M., & Zerbini, L. F. (2016). Novel potential serological prostate cancer biomarkers using CT100+ cancer antigen microarray platform in a multi-cultural South African cohort. *Oncotarget*, 7(12), 13945.
- Suwarnalata, G., Tan, A. H., Isa, H., Gudimella, R., Anwar, A., Loke, M. F., & Vadivelu, J. (2016). Augmentation of Autoantibodies by Helicobacter pylori in Parkinson’s Disease Patients May Be Linked to Greater Severity. *PloS one*, 11(4), e0153725.
- Liew, J., Amir, A., Chen, Y., Fong, M. Y., Razali, R., & Lau, Y. L. (2015). Autoantibody profile of patients infected with knowlesi malaria. *Clin Chim Acta*. 448, 33-38.

**Terms and conditions**

Sengenics Immunome Protein Array Slide - Contains over 1600 full-length, correctly folded and functional Human proteins, spotted in quadruplicate. Patented KREX functional proteomics technology which utilises the BCCP folding marker for the production of full-length, correctly folded and functional proteins. Protected by the following patents: EP1203238, JP4730804, GB2361698, US7816098, EP1470229, AU2003238441, US8999897, JP4377242, CA 2474457, EP1485411, CA2518927C, EP1456668, AU20032352355, JP4781628. Trademarked in the United Kingdom UK00003167383 under classes 05, 10 and 16. Except as otherwise agreed to by us in writing, the purchase of Products from us only conveys to you the non-transferable right for you to use the quantity of Products purchased in compliance with any applicable limited use statement or limited label license, as detailed in our catalogues, on our website, or on the label or other documentation accompanying the goods (all such statements or licenses being incorporated herein by reference as if set forth herein in their entirety). Unless otherwise authorized by us in writing, Products purchased from us may not be resold, modified for resale, or used to manufacture commercial products. All products and results from services are supplied / handed over by us to you on the condition that they may only be used by you alone (and no other third parties for and/or on your behalf) as instructed and directed in writing by Sengenics for your own internal, non-commercial and non-revenue and non-fee generating research purposes only. They are not in any circumstances to be used for drug or diagnostic purposes, nor are they intended for use in or on humans. By accepting delivery of our products or services, you are expressly agreeing to use our products or services for internal, non-commercial and non-revenue and non-fee generating research purposes only as specified in this paragraph. Products are not to be repackaged or resold and results from services are not to be used for any purpose apart from the research purposes specified in this paragraph. Any non-research use requires an ITAP license, the cost of such license is based upon the type of application of any Sengenics technologies, products or services for any purpose other than the internal, non-commercial and non-revenue and non-fee generating research purposes specified in this paragraph. You represent and warrant to us that the Products sold to you (i) will be used only for your own internal research, (ii) will only be used in compliance with any applicable limited use statement or limited label license or applicable law and (iii) will not be resold or otherwise transferred or conveyed to any third party. No license or immunity under any patent is either granted or implied by the sale of any of our Products except to the extent expressly granted in any respective label license or limited use statement (all such statements or licenses being incorporated herein by reference as if set forth herein in their entirety). You should evaluate whether your use of Products purchased from Sengenics requires permission or license from any third parties. Nothing in these Terms shall be deemed or construed (i) as a license or grant of any intellectual property, whether implied, by estoppel or otherwise except to the extent expressly granted under any applicable intended use statement, limited use statement or limited label license; (ii) to limit our rights to enforce our Intellectual Property, including, without limitation, as to use of any Product beyond that granted under any label license or statement applicable to the Products; (iii) as granting you any right to be supplied with goods or component thereof beyond those ordered by you and supplied by us in accordance with these Terms; or (iv) as a license or grant of any right to you to manufacture or to have manufactured the Products.

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