KREX Protein Arrays for Rapid Screening of Vaccine Candidates

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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 consistent, 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 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.

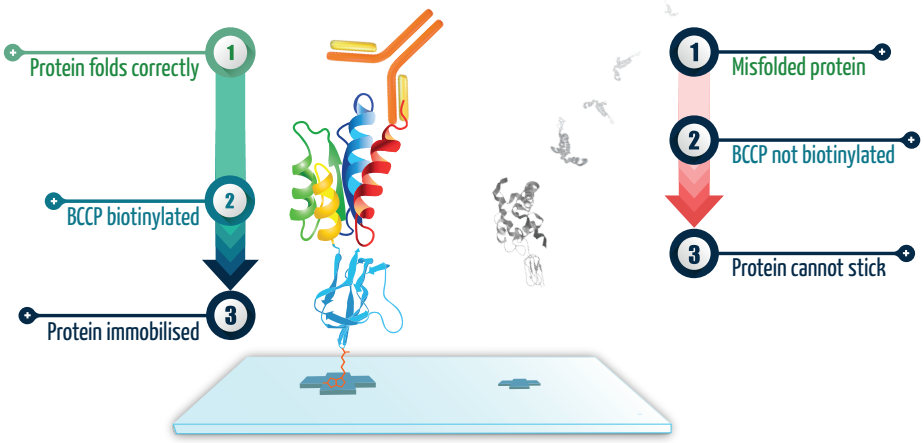


Figure 1: When the protein of interest is correctly folded, it co-translationally drives the correct folding of BCCP, which then becomes biotinylated and allows the fusion protein to become attached to the surface. On the other hand, when the protein of interest is grossly misfolded, it co-translationally drives misfolding of BCCP so it can no longer become biotinylated, preventing it from attaching to the surface. These proteins simply get washed away.

Introduction

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 host 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 sera and antibody interactions. Such an array can allow simultaneous examination of the magnitude of antibody responses, the isotype of such antibodies and the breadth of the bacterial/viral strain recognition.

Vaccine screening case study

Study Design:

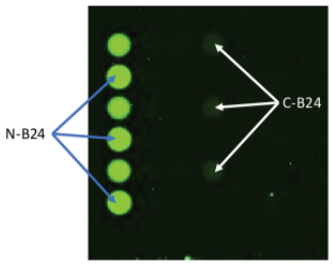
Bacterial meningitis is caused by *Neisseria meningitidis*, and evades the immune system by binding via its factor H Binding Protein (fHBP) to complement factor H. To identify potential novel meningitis vaccines, we fabricated a custom KREX protein array containing four recombinant N- or C-terminal, BCCP-tagged, fHBP variant proteins: A05, A15, B01 and B24. JAR4 and JAR5 meningitis monocolonal antibodies were assayed at various concentrations to obtain binding curve kinetics data. Sera collected from rabbits immunised with two protein-based vaccines (Tumenba®-Pfizer, Bexsero®-GSK) were also assayed on the arrays. A full description of the samples used in this experiment are shown in the table below:

Name	Description	Commercial Source
JAR4	Anti-Meningococcal factor H binding protein variant 1 (JAR4) monoclonal antibody	**NIBSC**
JAR5	Anti-Meningococcal factor H binding protein variant 1 (JAR5) monoclonal antibody	**NIBSC**
RS-T	Serum collected from rabbit immunised with MenB-FHbp Meningococcal Group B Vaccine	**Tumenba®, Pfizer**
RS-B	Serum collected from rabbit immunised with MenB-F4C Meningococcal Group B Vaccine	**Bexsero®, GSK**
IST1	Serum collected from rabbit with no immunisation (Negative Control)	**Not Applicable**

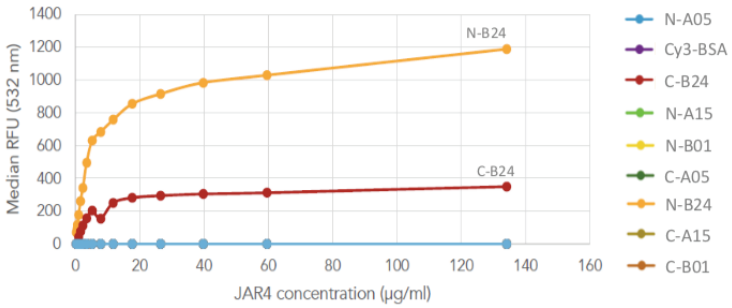
Results:

Kinetic data indicated that both JAR4 and JAR5 bind N- and C-terminally tagged variant B24 in the nanomolar range, as expected for monoclonal antibodies (Figure 2A and 2B). Furthermore, Tumenba® (Pfizer) shows high affinity to variants B01 and A05, producing K_d-values in the low-to-mid nM-range; whereas Bexsero® (GSK) shows a high affinity to variants B01 and B24 with K_d-values in the low nM and µM range, respectively (Figure 2C and 2D). As expected, the negative control sera (RS-Ctrl) showed no binding towards any of the fHBP variants (Figure 2E).

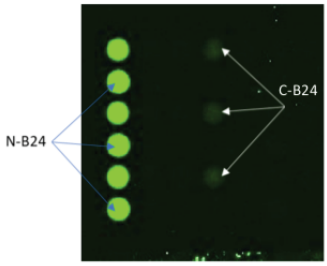
A:



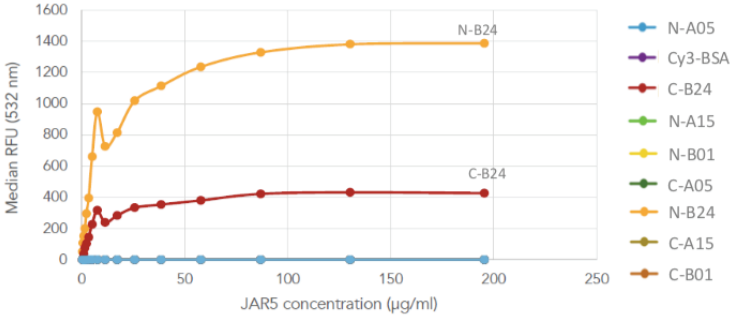
Microarray image of JAR4 (59.62µg/ml) assay showing binding to variant B24



B:



Microarray image of JAR5 (59.62µg/ml) assay showing binding to variant B24



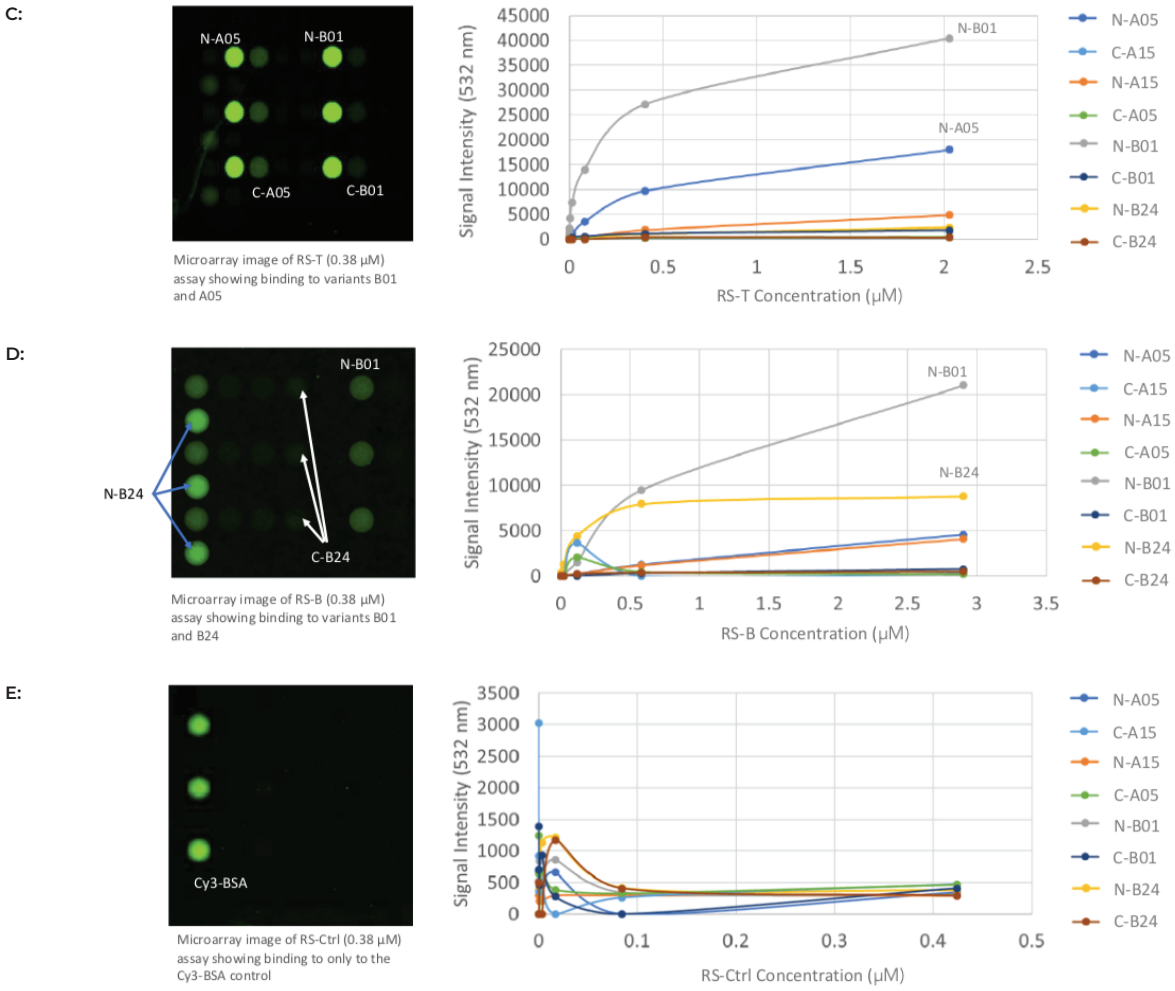


Figure 2: Protein array images and binding curves for all samples assayed across the Sengenics fabricated KREX protein array containing 4 recombinant N- or C-terminally tagged factor H binding proteins (fHBP); **A** & **B**: Protein arrays incubated with known meningitis monoclonal antibody vaccines, varying concentrations of JAR4 and JAR5 showed binding towards C- and N-terminally tagged variant B24 only; **C**: Protein arrays incubated with rabbit serum, RS-T at different concentrations, showed binding towards C- and N-terminally tagged variants A05 and B01; **D**: Protein arrays incubated with rabbit serum at different concentrations, RS-B, showed binding towards C- and N-terminally tagged variants B01 and B24; **E**: Protein arrays incubated with negative control rabbit serum at different concentrations, RS-Ctrl, showed no binding towards any of the fHBP variants on the arrays.

Conclusion:

The study has successfully demonstrated the use of KREX for the development of a highly sensitive and specific platform containing variants of factor H Binding Protein (fHBP) as a potential tool in screening and identifying vaccine candidates against meningococcal disease. The results from the study have shown the expected differential binding of the different “vaccine candidates” (monoclonal- and polyclonal- antibodies in purified and sera forms, respectively) towards the different variants of fHBP immobilised onto the surface. Moreover, we demonstrated the ability of the platform to produce detailed mechanistic properties for each of the “vaccine candidates” probed on the arrays, which is an advantage over existing screening methodologies.