Design and Functional Analysis of ssDNA Directed Assembly in Protein Array

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Abstract

In the post genomic era, the characterization of complex cellular functions requires the large scale analysis of the proteome. Antibody microarray technology has therefore become an invaluable tool in understanding the level of protein expression as well as protein-protein interaction in a high throughput manner. However, current fabrication approach of antibody microarray often results in the loss of antibody functionalities after immobilization onto the substrate. The present poster discusses the fabrication and evaluation of a new platform called "Spatially addressable protein array" (SAPA). By exploring the specificity of DNA hybridization, ssDNA-antibody conjugates would capture the antigen from complex biological samples in milieu and spatially addressed to specific location on the oligo array for detection. Such approach allows the protein-analyte interaction to take place in a solution phase and reduce the loss of antibody functionality due to unfavorable surface protein interactions. Further optimization has been done by investigating surface chemistry, non-specific protein adsorption and facile preparation of the ssDNA-conjugated antibody. Experimental studies have shown that the platform is able to detect samples at pM scale and it could be fine-tuned to achieve an optimal system for solving biological problems.

Introduction

During the past decade, amazing breakthroughs in genomics and proteomics created a great demand for miniaturized, high throughput platforms that allows robust collection and analysis of biomolecular data. Today, DNA microarray has become the mainstay technology for profiling the transcriptome in a cell. However, since the global protein complement determines the physiological state of the cell, antibody microarrays has emerge as the key technology for profiling the level of protein expression and an invaluable tool in disease diagnostics. Like any other protein microarrays, a significant challenge for protein chips fabrication is the selection of suitable surface matrix for protein immobilization. Ideally, the substrate should allow robust attachment of all the proteins at high densities without denaturing their conformation. Antibodies have been found to suffer from loss of activity and even have altered specificity after immobilization onto the surfaces. It is also important that the surface would be resistant to non-specific protein adsorption which would increase the background noise significantly. The present study therefore investigated a new platform called "Spatially addressable protein array" (SAPA) that circumvents the undesirable protein-surface interactions. The antibodies tagged with single stranded DNA would capture the antigen from complex biological samples in milieu and spatially addressed to specific location on the oligonucleotide array for detection. The probes could be conveniently stored in the solution phase which extends the shelf life of the array tremendously. It is also likely that the kinetics of binding between the antibody probe and cognate antigen in the homogenous phase would be greater than the heterogeneous solid liquid interactions in the conventional protein microarray platform. Other factors in the fabrication of a robust protein microarray were also analyzed: surfaces modified with carboxyl terminated dendrimer molecules to promote the immobilization of probes and reduce nonspecific protein adsorption. Suitable blocking reagents to reduce the background noise. Effect of concentration ratio between probe:analyte in optimizing the array performance. Our experimental studies proved that we could identify and discriminate samples at a 1 pM scale. Such versatility could allow identification of numerous target proteins based on the unique oligonucleotide addressable antibodies.

Our Strategy

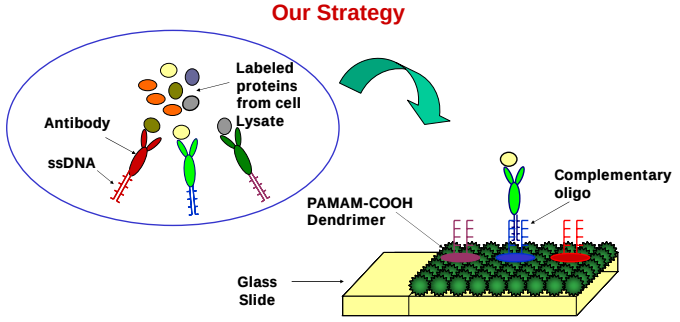

Figure 1. The schematics of the oligo tagged antibody capturing antigens and its spatial addressability through specific hybridization onto complementary oligonucleotides immobilized on PAMAM carboxyl derivatized glass surfaces.

Criterion for an ideal surface for protein microarrays

1. **The surface is inherently inert and resist nonspecific protein adsorption**
2. **It contains high density functional groups for the facile immobilization of probes**
3. **The linking chemistry allows the control of protein orientation and the local chemical environment favors the immobilized protein molecules to retain their native conformation**

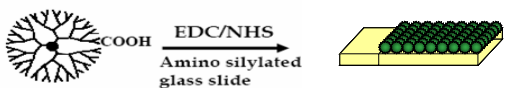

Figure 2. Schematic representation for the direct preparation of PAMAM-carboxyl dendrimer slides

Non Specific protein adsorption over different functionalized slides

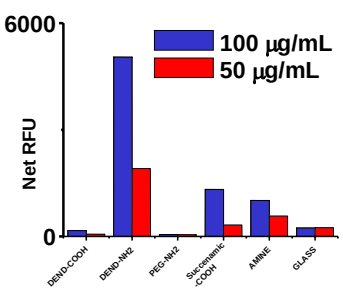

Figure 3. Nonspecific adsorption of the Cy5 labeled cell lysate over different functionalized slides

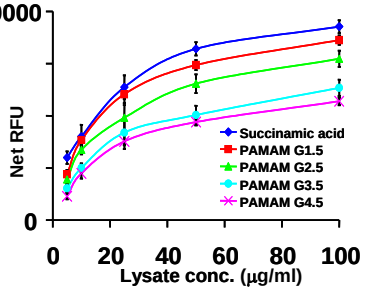

Figure 4. Nonspecific adsorption of the Cy5 labeled cell lysate over different generation of PAMAM carboxyl and succenamic acid derivatized surfaces

PAMAM carboxyl dendrimer coated slides is highly resistant to non-specific protein adsorption and is comparable with PEG coated slides. In addition, surfaces coated with higher generation of PAMAM carboxyl dendrimer is more resistant to protein adsorption due to the higher density of COOH group present on the surface.

Preparation of the immobilized address over the functionalized slides and comparison of the density of the functional groups

The immobilized address was prepared by covalently linking amino-terminated oligonucleotides with EDC/HNSA activated carboxyl dendrimer glass slide

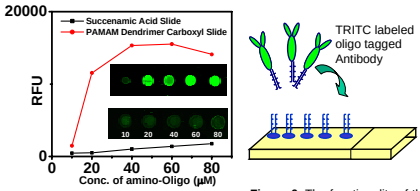

Figure 5. The density of the immobilized address- oligonucleotide over PAMAM carboxyl and succenamic acid slides were investigated by the hybridization of Cy3 labeled oligo over the glass slides.

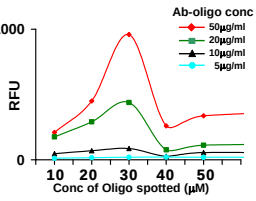

Figure 6. The functionality of these addresses were studied by the hybridization of an TRITC antibody tagged with complementary oligonucleotides. The amount of antibody captured on the dendrimer slides is proportional with the concentration of antibody-oligo conjugate in the solution. Optimal hybridization of probe with spotted oligonucleotide address at 30µg/ml, suggest that there is a crowding affect that reduces the efficiency of capture.

Capturing of rabbit IgG with Swine anti Rabbit IgG-oligo conjugate

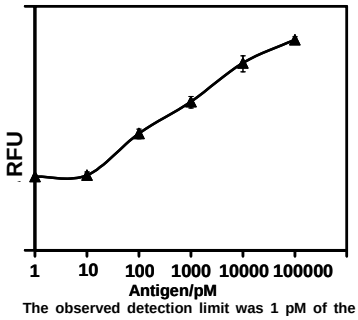

Figure 7. Different concentrations of antigen-rabbit IgG captured with 20 µg/mL ssDNA-Swine anti Rabbit IgG onto 30 µM oligo spotted slide.

The observed detection limit was 1 pM of the antigen with the signal-to-noise ratio of ~30. This shows the efficiency of the new approach of SAPA in the detection of low amounts of antigen (1 pM) with significantly lower concentrations of the capture reagent (0.1µM)

Summary

- **A new platform for protein microarray called "Spatially addressable protein array" (SAPA) has been designed, fabricated and investigated its functional role in antibody protein array.**
- **The newly developed platform has minimal nonspecific adsorption with high density compared to the existing systems**
- **The preliminary results showed that molecules in picomolar scale can be detected using this newly developed system**

References

[1] P. K. Ajikumar, N. Jinkiat, J. Y. Lee, H.P. Too, "**Role Of Surface Chemistry In Protein Chip: A Rational Approach For An Ideal System**", *ACS, National Meeting San Diego, March 2005.*(Accepted for presentation)
[2] P. K. Ajikumar, N. Jin Kiat, G. Stephanopoulos J. Y. Lee, H.P. Too, "**Spatially Addressable Protein Array (SAPA): A novel platform for antibody microarray**" (Manuscript under preparation)