Ultra-high Density Hydrogel Nanoarrays

A versatile platform for large scale proteomics

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Introduction

Despite the similarities to DNA microarrays, protein microarrays bring significant new challenges. Perhaps foremost among these is the limiting quantities of reagents available for array production and processing. We present here the development of a novel nanohydrogel substrate for the production of nano-scale protein arrays.

Materials and Methods

Nanoarray Fabrication

Glass microscope slides were silane treated and spin-coated with amine-terminated poly(ethylene glycol) (PEG-NH_2). Nanohydrogels of approximately 200 nm in diameter were generated using focused electron beams in a modified LEO-982 field emission scanning electron microscope (FEG-SEM). Approximately 100 mm diameter spots were created containing 7500 nanohydrogels per spot. Zinc Finger 9 [ZNF9] (Genbank NP_003409) was expressed as a GST-fusion protein. Two methods of protein attachment were explored. In the first, GST-ZNF9 along with negative control proteins were directly crosslinked to the nanohydrogels. In the second approach an anti-GST antibody was crosslinked to the nanohydrogel and used to capture the GST-ZNF9 protein.

Microarray Fabrication

Microarrays were printed with GST-ZNF9, anti-GST and negative control proteins on Schleicher & Schuell FAST slides, Corning Epoxide slides and Corning Ultra-GAPS slides using a GeneMachines Omnigrid 100 Arrayer (Genomic Solutions) with SMP3 pins (Telechem) yielding spots of 100µm diameter.

Hybridization and Detection

A cy5-labeled 55-mer oligonucleotide termed the Universal Substrate [US] was used to assess the nucleic acid binding activity of ZNF9. The US is predicted to form several different secondary structural features to bind to a variety of nucleic-acid binding proteins (NBPs). Slides were incubated with the US for 6 hours, rinsed, dried and scanned using a GenePix 4000B scanner (Molecular Devices) and the GenePix 5.1 software.

Results

Figure 1. Characterization of Nanoarrays. Nanohydrogel arrays were created and exposed to fluorescein isothiocyanate (FITC) overnight. The fluorescence of each 100 mm spot is easily measured in the Axon scanner and the discrete hydrogels can be imaged with a standard fluorescence optical microscope.

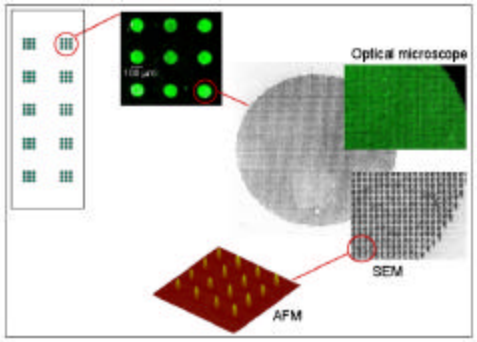


Figure 2. Fluorescent intensities of positive and negative control proteins. ZNF9, BSA, ßGal and GST were directly coupled to the nanohydrogels, or where indicated, the anti-GST antibody was coupled to the nanohydrogel and used to capture GST-ZNF9 and negative control proteins. The nucleic acid binding activity of the proteins was then interrogated using the cy5-universal substrate.

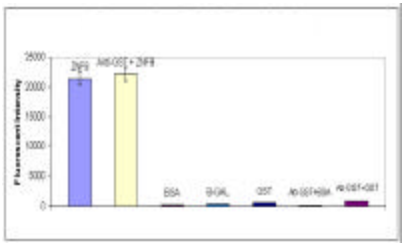
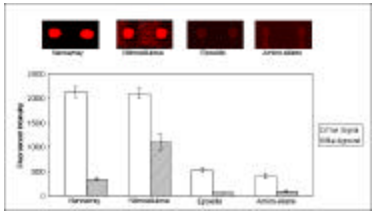
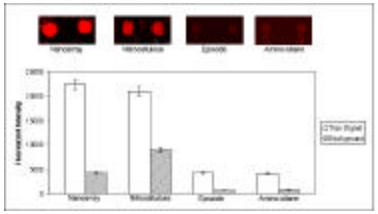


Figure 3. Nanohydrogel array outperforms three different microarray substrates in the NBP binding assay. (A) GST-ZNF9 was covalently coupled to the nanohydrogels. (B) An anti-GST antibody was covalently coupled to the nanohydrogels and used to bind GST-ZNF9. Both slides were interrogated using Cy5-labeled US.

A



B



Conclusion and Future Directions

In this study we demonstrated the feasibility of using nanohydogels for protein arrays. Though we are focusing on a nucleic acid binding assay to demonstrate the promise of this novel platform we can envision a number of applications for the hydrogel nanoarray where there is limited sample including: Screening urine or blood for protein or nucleic acid markers of disease; screening for autoantibodies; functional protein assays such kinase activity; screening libraries of proteins to determine function of unknown proteins; examining RNA levels small numbers of cells without RNA amplification; examining protein levels in 100 cells or less from laser capture microdissection of tumors. Our long term goal is to develop methods to deliver proteins to each individual nanohydrogel, thus permitting the interrogation of thousands of proteins in a 100 mm spot.