Exploration of Protein Capture Methods for Applications in Microfluidic Devices

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Abstract:

On-chip protein immunoprecipitation is often a substantial limitation in the continued development of microfluidic tools for detecting protein-protein interactions in high throughput. Though adaptable surface chemistry on glass and polydimethylsiloxane (PDMS) lend themselves to robust, on-chip protein immobilization, problems such as non-specific protein adsorption, rigorous washing conditions, and low protein synthesis yields readily diminish assay sensitivity in protein interaction screens. In an effort to improve on-chip protein expression levels, we explored various chip-protein conjugation methods, including antibody crosslinking via (3-aminopropyl)triethoxysilane-derivatized (APTES) surfaces, Protein A-conjugated surfaces, as well as streptavidin-biotin coated surfaces. Increasing sensitivity to expressed protein was also explored through varying concentrations of both capture and probe antibodies as well as through the adjustment of enzyme-conjugated or fluorescently-labeled probe antibodies. Through our protein-surface conjugation experiments, we are able to successfully synthesize and capture protein on-chip. Continued development towards quantifying the binding strengths of protein-protein interactions would mark a substantial advancement in cellular proteomics and may also help unveil new drug targets in human disease.

Introduction:

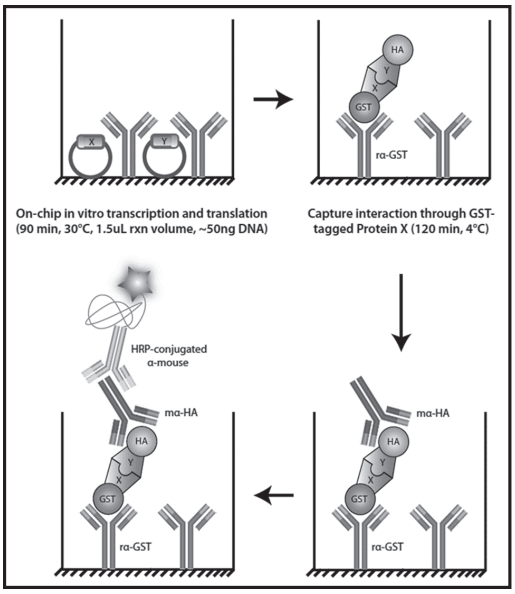
A longstanding goal in biological research has been to fully uncover the function of proteins encoded by human genomes as well as how their expression is regulated. Consequently, researchers have been working adamantly towards the development of high-throughput systems for expressing proteins and detecting their interactions under the paradigm that the function of a protein can be better understood by knowing who its interaction partners are. Though many well characterized high-throughput methodologies for detecting protein-protein interactions exist, including yeast two-hybrid and nucleic acid programmable protein array, these methods often suffer from limited specificity and high expense [1].

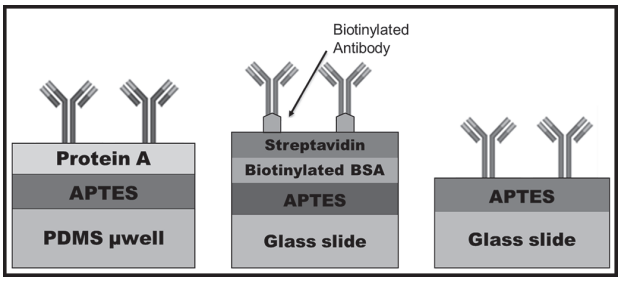
Microfluidic approaches such as Protein Interaction Network Generator demonstrate promise towards improving specificity and throughput but, due to difficulties in expressing and isolating human proteins through cell-free expression systems, have not been used to screen for human protein interactions on a large scale — a gap we aim to address [2]. The ability to quantify and generate protein-protein interactions networks could, in consequence, improve our understanding of how human disease alters cell signaling networks and potentially unveil new pharmaceutical drug targets.

Experimental Procedure:

Glass microscope slides underwent an oxygen plasma treatment before a 5 min bath in a 5% APTES/acetone solution. The slides were then rinsed with acetone then deionized water and dried with nitrogen gas. For Protein A-treated PDMS microwells, a 10:1 ratio of polymer to crosslinker (Silgard 184 Elastomer) was poured onto 500 μm tall SU-8 columns and cured for at least 2 hrs at 60°C. Slides are then crosslinked to protein using 2 mM bissulfosuccinimidyl suberate (BS3) and then quenched in 2M Tris and 0.3M glycine for 20 min. Next, 20 μL TnT® Reticulocyte Lysate is spotted on-chip containing 0.5 μg of protein pairs expressed in pANT7_nHA and pANT7_cGST vectors. This solution is incubated for 1.5 hrs in a humid environment at 30°C.

Following incubation, TnT® solution-coated slides were moved into a 4°C environment under high humidity for 2 hrs. After incubation, slides were washed in 1× PBS with 0.1% Tween-20 (PBST) three times for 5 min per wash. Anti-GST mouse antibody (Cell Signaling Technology, #2624) was applied at 1:500 concentration, followed by 1 hr incubation at room temperature in a humid environment. Slides were then washed in PBST three times for 5 min each. HRP-conjugated anti-mouse (Cell Signaling Technology, #7076) secondary antibody was





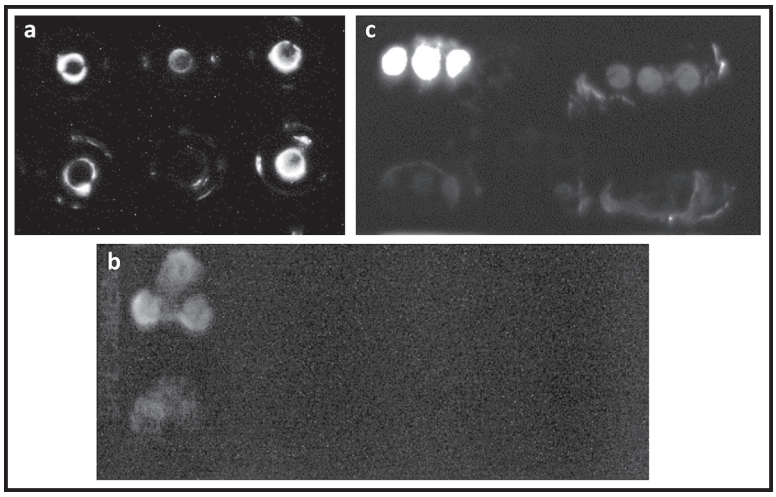


***Figure 1**, above: An overall schematic of the final device. **Figure 2**, top right: Three different protein surfaces tested for protein capture. **Figure 3**, bottom right: a. GST capture results from a Protein A surface. b. GST capture results from a biotin-streptavidin surface. c. GST capture results from an APTES only surface.*

then incubated for 1 hr at room temperature in a humid environment. Next, slides were washed with PBS three times for 5 min each. ECL substrate (GE, RPN2232) was then applied and slides were imaged for up to 10 min using a Bio-Rad ChemiDoc system.

Results and Discussion:

The APTES coated surface with direct application of antibody was the most effective, validated with high specificity when probing for the capture antibody alone. On the Protein A surface, false positives were observed as seen in Figure 3a. In Figure 3a, the rightmost column of luminescing points is composed of a positive control for GST capture on top and a negative control for GST capture on bottom. However, these points are indistinguishable from each other, and probing for GST capture on this protein surface did not yield conclusive results.

The biotin-streptavidin surface had less luminescence than APTES alone when probed solely for capture antibody, as observed in Figure 3b. The leftmost column is a positive control for GST capture; this luminescence was distinguishable from the negative control for GST capture in the rightmost column. The APTES-alone surface was able to repeatedly detect purified protein amounts as low as femtograms of protein as seen in Figure 3c. In Figure 3c, the upper left set of points is the positive control for GST while the negative control is in the middle of the figure. The positive and negative controls are clearly distinguishable from one another. Also, the lower left portion of Figure 3c illustrates femtogram-levels of GST capture.

Future Work:

Moving forward, protein tagged for both capture and probe antibodies must be expressed on-chip. Testing a dual-tagged protein will ensure that the antibodies in this procedure are valid to thoroughly measure interaction strength between two proteins. Once the final device is constructed and capable of quantifying tens of thousands of protein-protein interactions at a time, changes in protein-protein networks as a result of environment changes (introduction of drugs, pH, etc.) could be rapidly modeled and evaluated.

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References:

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