Label-Free Analysis of Protein Arrays

SPRimager®II analysis of arrays prepared on a Continuous Flow Microspotter

Introduction

The SPRimager®II is a label-free array detection system designed for rigorous, quantitative analysis of molecular interactions. Arrays for analysis on GWC's SPRimager®II system are typically fabricated by spotting sub-microliter volumes of ~0.1 – 1 mg/mL probe solutions onto array substrates (chips). However, when only dilute probe solutions are available, direct spotting of small volumes may not deposit a sufficient concentration of probe molecules on the chip to detect analytes in subsequent analyses.

Using Continous Flow Microspotting (CFM), arrays are fabricated by continuously flowing probe solutions over the array substrate. During CFM, greater densities of probe molecules can be deposited on the chip than can be achieved by traditional spotting methods with dilute probe solutions. CFM is therefore particularly well suited for experiments that involve immobilizing molecules out of a low concentration solution.

This note details use of GWC's SPRimager®II in conjunction with the Wasatch CFM system to fabricate and analyze protein arrays printed from low concentration probe solutions. The results show that CFM printing using as little as 2µg/mL protein solution generates protein arrays suitable for analysis on GWC's SPRimager®II system, and suggest that much lower probe concentrations may be successfully used in some cases.

The SPRimager®II and SPRchip™ substrates used were the standard products as provided by GWC Technologies Inc. The Wasatch CFM system used was a beta version kindly provided by Wasatch Microfluidics (North Salt Lake, UT).

Array fabrication and data collection

A three-component array was prepared by printing streptavidin (SA), β2microglobulin (β2m) and Human Serum Albumin (HSA) protein probes at 0-25 µg/mL on an NHS-activated SPRchip™. The probes were cycled across the surface for 40 minutes at 60µL/min, then washed for 5 minutes in PBS. The chip was then removed from the CFM system and dried in air.

The chip was mounted in the SPRimager®II and an initial image was collected in buffer (Fig. 1). The array was then blocked by washing with 1mg/mL BSA. Although little difference is evident between the probe spots prior to blocking, BSA bound to a noticeably greater extent (i.e. reflectivity increased most) where lower probe concentrations were spotted (Fig 2), consistent with probe concentration on the array varying as a function of concentration spotted.

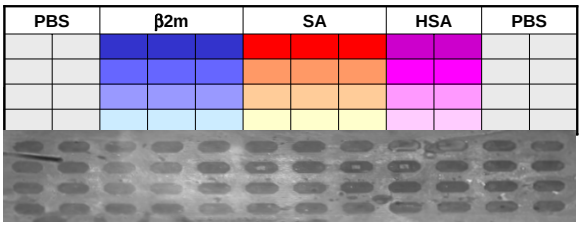


Fig 1: Initial image of the array. Top, Key to probes—grey, PBS, blue, streptavidin; red, β2-m; purple, HSA (25, 10, 5 & 2 µg/mL, top to bottom, for all probes). Immediately above, array image prior to exposure to any interacting molecules.

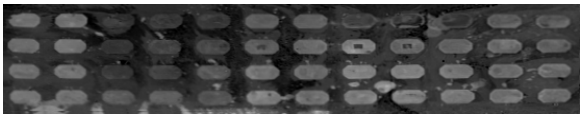


Fig 2: Blocking the array with BSA: the amount of BSA bound is inversely proportional to the probe concentration spotted (see Fig. 1 for key to probes)

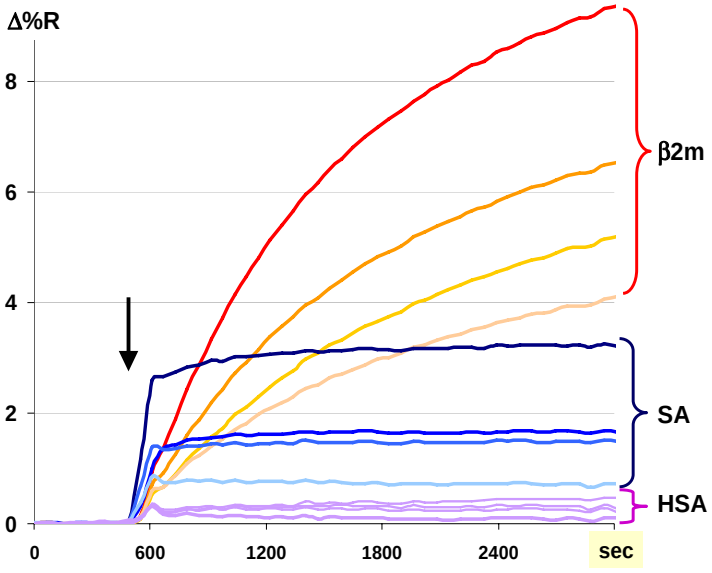
The array was then exposed consecutively to:

1. PBST buffer, to establish a baseline;
2. Biotinylated anti-β2-microglobulin antibody (anti--β2m, polyclonal; Rockland) at 20µg/mL, which should bind to its antigen β2-microglobulin and to SA;
3. Buffer, to wash away nonspecifically bound protein;
4. Anti-HSA antibody (anti-HSA, mouse monoclonal, Pierce) at 20µg/mL which should only bind to its antigen, HSA;
5. Buffer.

Both image and numerical data were collected in real time on the SPRimager®II for the entire sequence of exposures to the different analytes. The images allow the user to monitor visually the extent of binding to each spot throughout the experiment. For data analysis, SPR signals were first converted to absolute reflectivity changes Δ%R. Next, replicate spots for each concentration were averaged. Finally, averages were corrected for background by subtracting the control PBS spot signal averages.

Results

Time course of binding of biotinylated anti-b2m antibody to its antigen and to SA



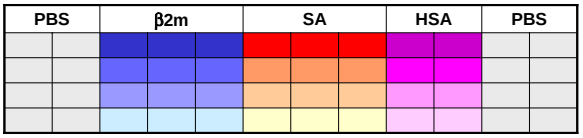
The chart at left shows the time course of binding of biotinylated anti-β2m to the array (color scheme same as Fig 1 key). The arrow indicates where the switch was made from flowing PBST buffer to flowing anti-β2m. Protein function is clearly maintained: the antibody bound rapidly to SA and slowly to its antigen, as expected. For both β2m and SA probes, the extent of antibody binding is higher where probe density is higher. Binding is nevertheless readily detectable for the lowest concentration of probe spotted here, 2µg/mL. For the β2m probe, useful arrays could surely be fabricated by CFM using probe solutions well below 2µg/mL.

Quantifying protein binding

To compare the extent and consistency of antibody binding to the various probe spots, difference images were obtained from which the reflectivity changes (ie extend of Ab binding) could be determined as follows:

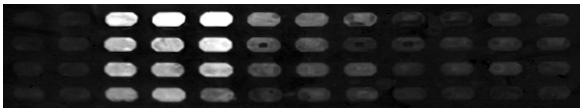
A. To assess binding of anti-β2m to its antigen β2m and to SA, the image collected at 427 seconds (prior to antibody addition) was digitally subtracted from the image collected at 3,439 sec (last image collected in the presence of the antibody);

B. To assess binding of anti-HSA to its antigen HSA, the image collected at 3,439 sec (i.e. terminal image from part 1 above) was subtracted from the image collected at 6,564 sec (last image collected in the presence of the anti-HSA antibody) (not shown).



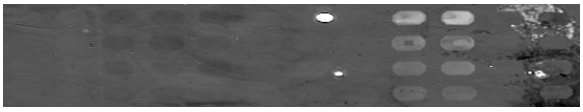
Top: array probe layout, color key same as Figure. 1.

A



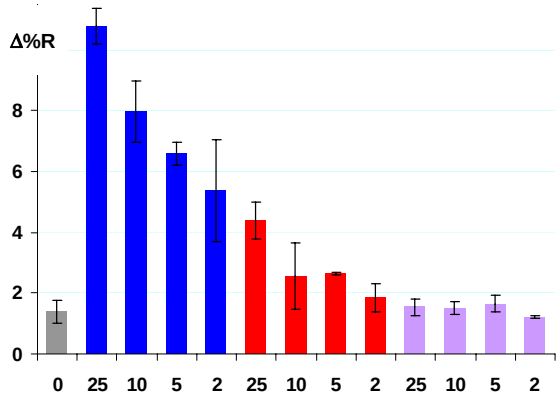
Difference image A illustrates the extent and specificity of the anti-β2m binding to β2m and to SA.

B



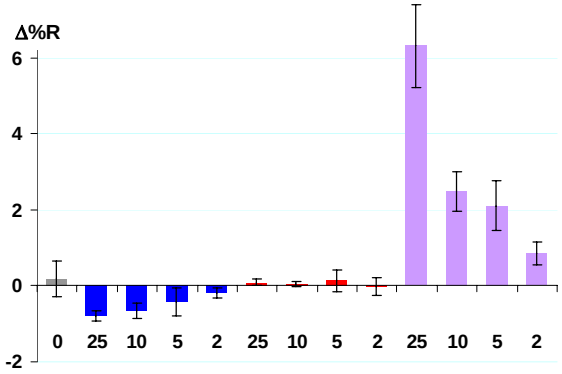
Difference image B illustrates the extent and specificity of anti-HSA binding to HSA.

C



Quantification of protein binding confirms the visual impression that more protein bound to probe spots that were printed at higher probe concentrations. In histogram C, binding is quantified for all probes—blue, β2m; red, SA; purple, HSA (numbers on x-axis indicate probe concentration spotted in µg/mL). Anti-β2m bound more to its antigen than to SA. Some nonspecific binding to HSA is observed though this is no higher than to PBS control spots.

D



Histogram D quantifies the binding of the anti-HSA to all probes. At this late stage in the experiment, after exposure to BSA blocking agent and the prior antibody, there is no significant nonspecific binding, and again, more antibody bound the higher the concentration of HSA probe printed. The negative values for β2m probes here reflect dissociation of the antibody from its antigen—subsequent exposure to anti-HSA is essentially a wash step for these probes.

Conclusions

Protein arrays suitable for analysis on the SPRimager®II system were created by CFM printing of probe solutions at concentrations of 2-25µg/mL. With CFM printing of dilute probe solutions, good probe density can be obtained on array surfaces by simply extending the duration of CFM printing. Moreover, for the β2m probes, even at 2µg/mL probe spotted, antibody binding was far above the minimum detectable, indicating that at least for some probe-target combinations, CFM spotting, concentrations in the ng/mL level should generate perfectly functional arrays.

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