Optimizing Carbohydrate Microarray Fabrication

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Statement of Purpose: The carbohydrate microarray is a powerful emerging tool for the field of glycomics – advancing our understanding of glycobiology by rapidly profiling the carbohydrate-binding profiles of proteins, nucleic acids, bacteria, viruses and cells. The glycan array is based on the presentation of carbohydrates on a solid substrate, such that they can be screened with glycan-binding analyte in solution. However, adoption of the microarray paradigm for glycomics research faces several challenges that need to be addressed before this technology can realize its full potential. One challenge is the propensity of fluid printed on a microarray to evaporate during the covalent or non-covalent attachment of carbohydrates to the surface, resulting in non-uniform array spots. In addition, the use of fluorescent probes is often a prerequisite for detection of glycan-binding partners. Binding experiments that require labels are not always practical (i.e. pre-purification and labeling of analyte is needed), and their sensitivity is dependent on the efficiency of the labeling.

To address these issues, we examined the role played by glycan array printing conditions (e.g. pH, incubation time, printing humidity, printing addictives) on microarray performance using a label-free biosensing technique (surface plasmon resonance, SPR). These findings demonstrate the potential of optimizing printing conditions for improving glycan array fabrication and result interpretation, and advancing the application of glycan array to biological systems and modeling.

Methods: Model amine-functionalized carbohydrates were prepared via the reductive amination of sugars with 2,6-diaminopyridine (DAP).[1] Microarray substrates bearing *N*-hydroxysuccinimide (NHS) and pentafluorophenyl (PFP) esters were subsequently used for immobilization of the amine-modified glycans. SPR was performed on a SPRimager®II system [2] (GWC Technologies) to study protein/carbohydrate specificity.

Results: To achieve high-throughput screening of the bioactivity of immobilized glycans, 100-element carbohydrate microarrays were fabricated for use in label-free SPR imaging studies. Utilizing a robotically printed 10 x 10 array of carbohydrate and glycoprotein immobilized on NHS- and PFP-functionalized gold surfaces, we monitored 100 carbohydrate/protein interactions simultaneously. A representative 100-element microarray and the corresponding sensorgrams resulting from protein binding are shown Figure 1. Theses arrays were further used to optimize the conditions of microarray printing conditions.

During array fabrication, nanoliter droplets of solution evaporate within seconds when the relative humidity is below 50%. This evaporative process results in non-uniform distribution of covalently-bound glycan within each array element. To demonstrate this phenomenon, and to establish how we could reduce the heterogeneity of the printing spots, we studied the effects of relative humidity and printing solution composition (e.g. pH, DMF, Tween-20) on microarray performance. (Fig. 2) SPR-binding studies on PFP surfaces showed a significant improvement in both spot size (uniformity) and the overall protein-binding capacity for spots printed at high humidity, high pH, and in the presence of surfactant.

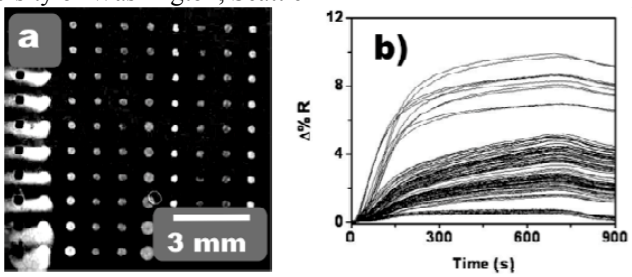

Figure 1. (a) A representative 100-element microarray and (b) the corresponding sensorgrams resulting from the lectin ConA.

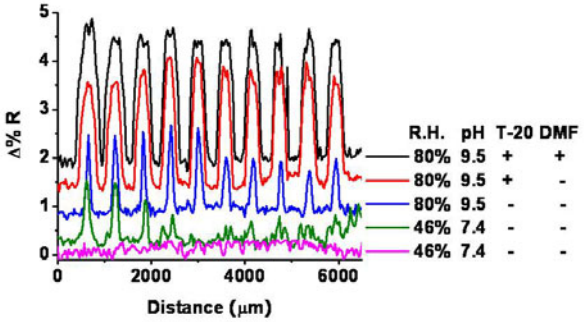

Figure 2. Line profiles of SPR reflectivity representing ConA binding under various printing conditions.

Conclusions: We compared the activity of two amine-reactive surfaces (NHS and PFP) and studied the role of printing conditions (e.g. pH, relative humidity, printing additives) on carbohydrate microarray performance and array quality. Printing buffers with a pH of 7.4 and 9.5 were found to be optimal for array substrates bearing NHS and PFP, respectively. High humidity and presence of surfactant aids spot spreading during array printing, improving the uniformity of the element and increasing protein binding capacity. The results from this study will subsequently be used to develop improved fabrication techniques and aid in the interpretation of glycan array results.

References:

[1] Xia, B., et al., Nat. Methods. 2005; 2: 845.
[2] Smith, E. A., et al., J. Am. Chem. Soc. 2003; 125: 6140.