Sweet Surfaces: Carbohydrate Microarrays and Tools for Glycomics.

Daniel M. Ratner* and Peter H. Seeberger¶
Boston Medical Center,* ETH Zürich.¶

Statement of Purpose:

The burgeoning field of glycomics has been stymied by a scarcity of biophysical and molecular tools. Detecting glycan interactions is cumbersome and carbohydrates have no biological amplification counterpart to the polymerase chain reaction (PCR) or recombinant protein expression. These limitations have hindered our study of glycobiology.

We propose that the union of synthetic chemistry and materials science will close this technological gap. Advances in synthetic carbohydrate chemistry, the development of an automated solid-phase oligosaccharide synthesizer,[1] and fabrication of solution-phase microreactors,[2] provide access to a growing number of glycans of increasing structural complexity. Utilizing glycans derived from these techniques, we have covalently immobilized synthetic carbohydrates to surfaces to create a variety of tools to study carbohydrate-protein, carbohydrate-nucleic acid, and carbohydrate-cell interactions. These methods are poised to enable a new generation of high throughput studies in glycomics.

Methods:

Synthetic glycans for this study were prepared by a linear solution-phase strategy employing trichloroacetimidate glycosyl donors.[3] For the purposes of covalent immobilization, a mercapto-PEG linker was incorporated into the reducing end of the carbohydrate.[4] Modified surfaces and neoglycoproteins were fabricated using standard thiol chemistry.[5] In summary, Corning® GAPS II slides (Corning, NY) were modified with SMCC (Pierce Biotechnology Inc., Rockford, IL) to display maleimide functionality. Thiol-modified glycans were printed onto the surface using standard robotic microarray technology and unreacted maleimide was quenched with 2-(2-(2-mercaptoethoxy)ethoxy)ethanol. Following incubation with fluorescently-labeled analyte (protein, nucleic acid, bacteria), the slides were washed with buffer and read by a fluorescent microarray scanner.

Results / Discussion:

HIV Glycobiology was selected for a preliminary study to demonstrate the new microarray platform. Glycan-dependent HIV host-pathogen interactions are well characterized and the antimicrobial proteins Cyanovirin-N (CVN) and Scytovirin were found to block HIV invasion by binding viral-associated carbohydrate. Establishing the mechanism of HIV inhibition by CVN and Scytovirin could be useful towards the development of these and other HIV prophylaxes.

Microarrays composed of the binding epitopes of the N-linked high-mannose oligosaccharide (Man_9) were synthesized as previously described.[3] In addition, the viral envelope glycoproteins gp120 and gp41 were immobilized on amine-reactive slides to control for peptide dependent interactions. HIV-binding proteins Cyanovirin-N, Scytovirin, CD4, DC-SIGN and human monoclonal antibody 2G12 were screened for carbohydrate affinity.[6] These results identified unique binding specificities for Cyanovirin-N and Scytovirin (Figure 1), established that binding of these proteins is insensitive to the peptide context of glycan display, and provided targets for an effort to develop a synthetic vaccine against HIV.

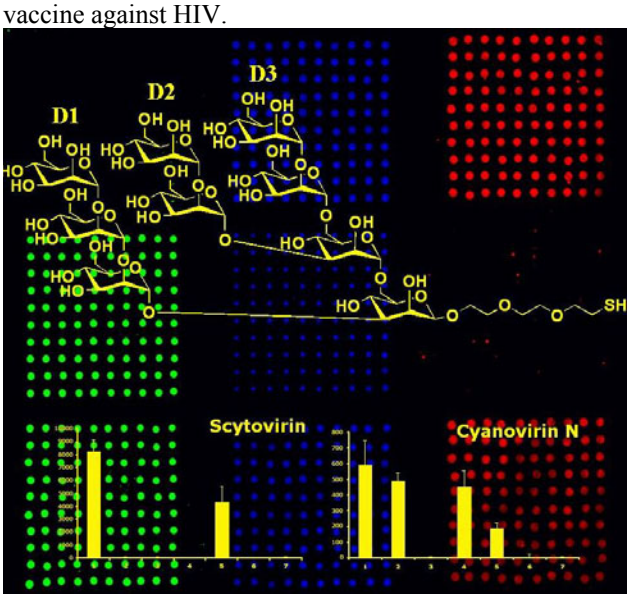

Figure 1. Microarrays of synthetic carbohydrates facilitate rapid identification of carbohydrate binding events.

Encouraged by the success of the initial microarray study, the chip-based method has been expanded to include: aminoglycosides to study antibiotic resistance, whole-cell binding of bacteria to identify carbohydrate-specificities of enteric pathogens, new chemistries for the immobilization of isolated glycans, and a malarial glycosylosphatidylinositol (GPI) microarray to facilitate the development of a synthetic carbohydrate-based vaccine against malaria.

Conclusions:

Carbohydrate microarrays have successfully achieved assay miniaturization and high throughput screening for glycan interactions – reducing the amount of carbohydrate and analyte required for study, and speeding analyses by weeks over conventional methods. As synthetic efforts continue to advance, structural diversity of oligosaccharides available for microarray fabrication will grow. These microarrays stand to make significant contributions to glycomics and biomedical research.

References:

[1] Seeberger PH. *Science.* 2001;291:1523-1527.
[2] Ratner DM. *Chem. Commun.* 2005;5:578-580.
[3] Ratner DM. *Eur. J. Org. Chem*. 2002;*5*:826-833.
[4] Ratner DM. *ChemBioChem.* 2004;5:379-383.
[5] Ratner DM. *ChemBioChem*. 2005;5:1375-1383.
[6] Adams EW. *Chem. Biol.* 2004;11:875-881.