the crosslinked termini. This was cleverly done by introduction of a cleavage site for TEV protease downstream of the introduced cysteine residues. The crosslinked proteins were then digested with TEV to liberate the crosslinked peptides for purification followed by characterization by mass spectroscopy. The mass spectral analyses demonstrated that indeed the designed crosslinks between the KS domains had been formed, and thus the dimer must be composed of monomers in a head-to-head configuration. These experiments provide a platinum standard of crosslinking analysis.

The detailed FAS structure remains to be established, but the complementation data argue that the regions of the two subunits downstream of the KS domain are somehow coiled together and this coiling is required to hold each monomer in the proper conformation for catalysis. Recent cryo-electron microscopy data suggest that the FAS dimer has an extremely flexible H-shaped structure [14–16]. The present data argue that the KS domain makes up the cross-stroke of the H. Why did the head-to-tail model seem so reasonable (and why was head-to-head evidence missed) in the 1980s? I believe this was largely due to the weakness of the tools then available compared to the size and complexity of FAS. SDS gel resolution of large proteins was poor and mass spectroscopy of large molecules would have been only a dream. Despite this wrong turn, it seems unlikely to me that the incorrect head-to-tail model seriously impeded progress with FAS. The difficulties have been technical, rather than conceptual, and working with such large enzymes remains a formidable challenge.

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Catching Bacteria with Sugar

In this issue of *Chemistry & Biology*, Disney and Seeberger exploit bacterial targeting of host cell surface sugars during pathogenesis to create a simple diagnostic carbohydrate microarray for the detection of bacteria in complex biological mixtures [1].

Mammalian cell surfaces are coated by a layer of carbohydrate molecules attached to lipids and proteins known as the glycocalyx. These often complex and heterogeneous glycans are involved in a diverse array of biological processes including inflammation, metastasis, cell-cell adhesion, and pathogen-cell interactions [2, 3]. The complexity of carbohydrate epitopes and the general lack of methods for characterization of protein-glycan interactions in a rapid and systematic manner have impeded our understanding of glycosylation. Recent progress in the area of array technology, specifically the development of carbohydrate microarrays, has changed dramatically our ability to assess carbohydrate-protein interactions at the molecular level. In general, microarrays allow for the analysis of a series of interactions simultaneously, thus providing a probe-based profile of a sample. Microarrays also present ligands in a multivalent manner, an important consideration for the study of carbohydrate-protein interactions where avidity is crucial [4]. Several different types of carbohydrate arrays have been designed utilizing both naturally derived and synthetic glycans on media ranging from multiwell plates to modified glass slides [5–9]. Although work on carbohydrate arrays is still in its early stages, they have already been useful in characterizing the glycan binding of antibodies, lectins, and other carbohydrate binding proteins and in examining the adhesion properties of hepatocytes and leukocytes [6–11]. In this issue of *Chemistry & Biology*, Disney and Seeberger elegantly demonstrate a new application for carbohydrate arrays as a diagnostic tool for bacterial pathogens.

Bacteria are notorious for having a “sweet tooth,”

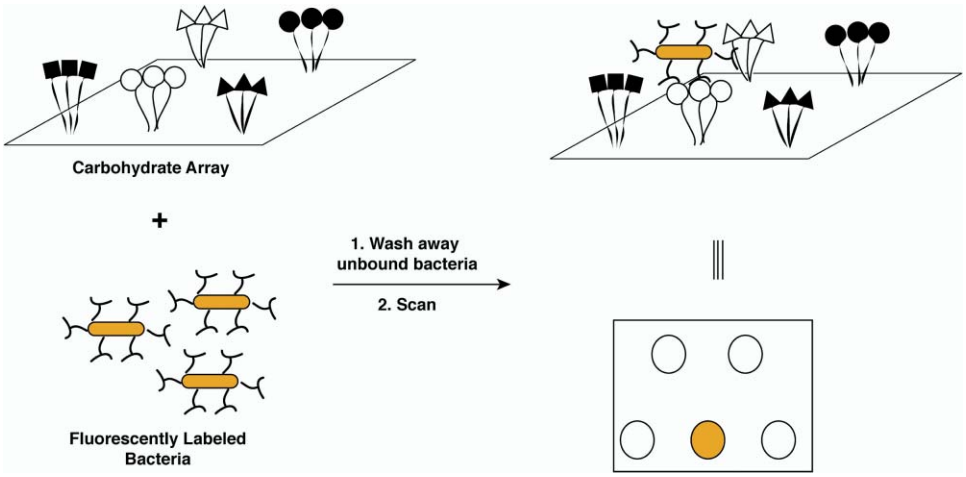

Figure 1. *Escherichia coli,* Fluorescently Labeled with a Nucleic Acid Staining Dye, Are Bound to a Carbohydrate Array

Unbound bacteria are washed away and the array is scanned resulting in a characteristic binding pattern based on sugar binding affinities. The multivalent nature of the carbohydrates on the array is indicated.

using the carbohydrates on mammalian cells to guide their invasion of the host organism [12, 13]. Protein-sugar interactions between the bacteria and the host cell are a crucial first step in the infectious process; thus, they provide a potential means of both diagnosis and treatment of bacterial infections. On page 1701 of this issue, Disney and Seeberger report the use of a simple carbohydrate microarray to detect bacteria in complex biological samples. In this initial work, a carbohydrate microarray was constructed using standard techniques and five monosaccharide derivatives, glucose, galactose, mannose, N-acetyl-D-glucosamine, and fucose. Even with such a basic array, specific binding of the fluorescently labeled *Escherichia coli* strain ORN 178 to mannose was observed (Figure 1). The array was also able to detect strain-specific differences in binding between ORN 178 and another strain of *E. coli*, ORN 209, that has reduced affinity for mannose. Perhaps the most striking aspect of this work however is the detection of bacterial contamination of a sample of sheep erythrocytes and serum by the addition of a fluorescent nucleic acid staining dye to the sample followed by direct binding to the array. The simplicity of this methodology opens up new possibilities for the detection and rapid characterization of bacterial strains.

Conventional methods for the typing of bacteria, such as plating and culturing, are time consuming, taking on the order of days to weeks to complete [14]. New technologies such as ELISA assays and PCR-based analysis have significantly reduced the timescale of detection but still require hours [15]. The work presented by Disney and Seeberger offers the possibility of rapid detection and characterization on the timescale of minutes. Two main issues will need to be addressed before such a system is viable however. First, can carbohydrate arrays be used to accurately type the wide variety of known bacterial strains? It is highly probable that the answer to this is yes. Differences in carbohydrate binding properties are widely believed to be responsible for the organ specificity and pathogenicity displayed by bacteria [12, 13]. Thus, binding of a bacterial strain to an appropriate library of carbohydrate residues in an array format could be expected to yield a glycan binding fingerprint, which could then be used to identify the bacteria in question. Given that in the work presented herein the spots on the array are $\sim$200 μm in diameter and that one slide can potentially hold thousands of different spots, a very detailed analysis of the carbohydrate binding affinity for a bacterial strain could be obtained. Of course a library of more diverse and complex saccharides will need to be arrayed, but this is well within the purview of the current carbohydrate array technology, as demonstrated by the more complicated mannosides tested against ORN 178 by Disney and Seeberger. In addition, identification of bacterial strains using this method could be immediately cross-checked using the more time consuming culture or PCR methods, as bacteria can be harvested from the carbohydrate arrays and then cultured as shown herein.

The second issue is that of detection limits. In the system demonstrated by Disney and Seeberger the limits of detection for bacteria are 10^5–10^6 cells. This limit is worsened when the bacteria are a contaminant in a more complex biological sample. Although, these limits are in line with other techniques currently in use, they are suboptimal as ELISA and PCR-based techniques can detect bacteria at 100–1000 cells/g of material [16]. It should be noted however that cultural enrichment of the material is typically required in those cases. Given advances in technology for both microchip readers and fluorophores, it is likely that comparable levels of detection using a carbohydrate microarray will be achievable with an optimized version of the system [17].

Beyond its utility as a diagnostic for bacterial contamination, bacterial binding patterns to a more complex glycan array could provide useful information for the creation of new strain-specific therapeutics. By adding to fundamental knowledge about the molecular interactions leading to pathogenesis, the use of such an array would aid the development of antiadhesive

agents to prevent bacterial infections. In addition, carbohydrate arrays can be used as a screen for such agents, as demonstrated in initial work by Disney and Seeberger in which they examine the relative inhibitory ability of three mannose-derived compounds.

The work shown herein by Disney and Seeberger is uncomplicated in its presentation. By using just five monosaccharides, standard array techniques, and fluorescently labeled bacteria, they elegantly demonstrate the potential of sugar microarrays for fast assessment of bacterial contamination. This simple study opens the way for the molecular dissection of very complex carbohydrate-bacterial interactions and for the use of such information to create both rapid diagnostic techniques and new therapeutic agents.

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