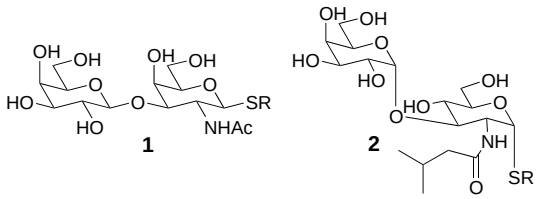
*Carbohydrate Recognition*

# Overcoming Degeneracy in Carbohydrate Recognition**

*Amit Basu and Daniel Kahne**

Protein binding to cell-surface carbohydrates is a key event in many important biological processes, including fertilization, development, inflammation, and cancer.[1] These events and the responses they trigger are difficult to study and to inhibit selectively because protein receptors for carbohydrates frequently have overlapping or degenerate binding specificities.[2] For example, the tetrasaccharide sialyl Lewis X present on endothelial cells and neutrophils binds to at least three different proteins, E-, P-, and L-selectin during the initiation of the inflammatory response.[3] For the study of recognition processes or events involving carbohydrate-binding proteins (lectins), it would be useful to have ligands that bind to one protein in the presence of other proteins that have similar binding specificities. Herein we show that it is indeed possible to find carbohydrate ligands that are specific for one among a group of carbohydrate-binding proteins that have the same nominal binding selectivity.

We previously reported the synthesis of a 1300-member combinatorial library of di- and trisaccharides on TentaGel beads.[4] This library was screened against *Bauhinia purpurea* (BP) lectin, which recognizes ligands terminating with the disaccharide Galβ1-3GalNAc (**1**, Scheme 1). We found that an unnatural carbohydrate (**2**) binds better to BP than all the other carbohydrates present in the library, which included the thioglycoside derivative of the natural ligand **1**.[4,5] This result showed that carbohydrate-binding proteins can discriminate one particular carbohydrate ligand among a large collection of related carbohydrates.[6] Furthermore, we have shown that the nature of the polyvalency of the ligand affects the selectivity of BP for **2** versus **1**.[5b] However, if unnatural carbohydrate ligands are to be used to probe carbohydrate–protein recognition processes, the binding specificity must be reciprocal; that is, not only must the lectin bind one particular carbohydrate ligand in the presence of related ligands, but the ligand in question must bind that protein selectively in the presence of other proteins that have the same nominal binding specificity.[7] It is not a forgone conclusion that a ligand selected in a screen will be specific for the protein used in the selection, particularly if the protein is known to be promiscuous with respect to natural ligands (see above). Therefore, the central question we sought to answer in this study is whether **2** would be specific for BP only, or whether it would exhibit some level of degeneracy in its interactions with lectins that bind **1**.




***Scheme 1.*** Structures of the natural (**1**) and hit (**2**) ligands attached to TentaGel resin. R = $SC_6H_4OCH_2CONH$TentaGel or photocleavable linker (see reference [13]).

To evaluate whether the binding selectivity of ligand **2** was reciprocal, we studied whether **2** could discriminate between the BP lectin and that from *Arachis hypogaea* (AH), which also recognizes ligands terminating with disaccharide **1**.[5b,8] We prepared TentaGel beads functionalized with **1** or **2** and incubated them with mixtures of BP and AH.[9,10] To facilitate detection of the proteins, BP was labeled with fluorescein (BP-F) and AH was modified with tetramethylrhodamine (AH-T). Fluorescein and tetramethylrhodamine have distinct fluorescence emission wavelengths, which allows the separate detection of both proteins on individual beads. The beads were incubated with the mixture of proteins and then examined with a confocal microscope.[11,12] Beads which were functionalized with ligand **1** had strong emissions at

[*] Prof. D. Kahne, Dr. A. Basu
Department of Chemistry
Princeton University
Princeton, NJ 08544 (USA)
Fax: (+1) 609-258-2617
E-mail: dkahne@princeton.edu

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the wavelengths for both fluorescein and tetramethylrhodamine, thus showing that both lectins were bound simultaneously to the beads (Figure 1). In contrast, the fluorescence from AH-T on beads containing **2** was barely above background levels, while the fluorescence from BP-F was very strong. Hence, ligand **2**, which was originally identified by screening a combinatorial library of carbohydrates for binding to BP lectin, binds that lectin almost exclusively, even in the presence of another lectin that has the same nominal binding selectivity.

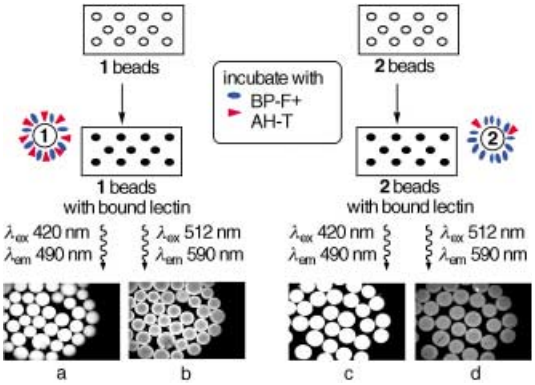


***Figure 1.*** Confocal microscope images of the binding of a solution of BP-F and AH-T to TentaGel Beads functionalized with **1** or **2**. a) Fluorescein emission from BP-F bound to **1**; b) tetramethylrhodamine emission from AH-T bound to **1**; c) fluorescein emission from BP-F bound to **2**; d) tetramethylrhodamine emission from AH-T bound to **2**.

These initial experiments showed that beads derivatized with ligand **2** were quite selective for the BP lectin over AH. We next sought to determine if we could use carbohydrate-derivatized beads to isolate BP lectin from a mixture of lectins which all recognize ligands terminating in disaccharide **1**. The ligands **1** and **2** were attached to beads using a photocleavable linker that can be cleaved under mild conditions to release carbohydrate-bound lectins into solution.[13] Lectins that are bound nonspecifically to the resin itself or that have denatured on the surface should not be released upon cleaving the carbohydrates.

In the event, carbohydrate-derivatized beads **1** and **2** were incubated with a solution containing a mixture of three lectins which all bind to ligand **1**: BP, *Agaricus bisporus* (AB), and *Vicia villosa* (VV).[14,15] After washing the beads, they were photolyzed to release the bound lectins into solution. The solution was filtered to remove excess albumin from the buffer and then the filtrate concentrated and analyzed by using denaturing gel electrophoresis followed by silver staining. The final results of this experiment are shown in Figure 2. The beads derivatized with the natural ligand bound all three lectins in similar amounts as shown by the presence of bands representing all three proteins in lane 1.[16] In contrast, beads derivatized with ligand **2** bound BP lectin almost exclusively (lane 2). Most importantly, this experiment demonstrates that unnatural carbohydrates can be used to extract proteins from a mixture with a selectivity suitable for subsequent analysis, such as sequencing. The preceding

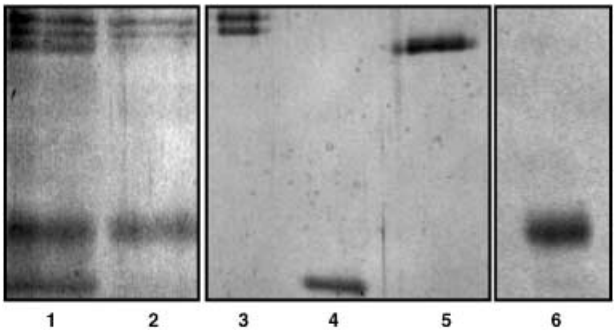


***Figure 2.*** Denaturing gels showing lectin release from TentalGel beads functionalized with **1** or **2** after photocleavage. Lane 1: lectins bound to beads containing **1**. Lane 2: lectins bound to beads containing **2**. Lane 3: BP lectin. Lane 4: AB lectin. Lane 5: VV lectin. Lane 6: avidin.

results illustrate that degeneracy in carbohydrate recognition can be overcome by the use of unnatural carbohydrates in place of the natural ligands.[17]

A specific carbohydrate ligand for that receptor was identified by screening a library of polyvalent carbohydrates against a particular protein receptor. This unnatural carbohydrate ligand bound avidly to the protein it was screened against, but not to other proteins, including proteins that recognize the same natural carbohydrate ligand. Carbohydrate-derivatized beads or surfaces have clear applications in protein isolation and extraction as well as in sensors for detecting carbohydrate-binding proteins.[5a,18] Recent developments in carbohydrate microarray technology, coupled with advances in oligosaccharide synthesis, will result in the identification of a large number of protein targets which bind and interact with oligosaccharides.[19] Perhaps more interesting, unnatural carbohydrate ligands that are specific for particular carbohydrate-binding proteins could be useful tools for probing biological processes or pathways involving monovalent or multivalent carbohydrate–protein recognition events.[20]

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[16] The avidin in lanes 1 and 2 is derived from the avidin-linked resin that was used to remove the biotinylated BSA present on the beads which prevented nonspecific lectin adhesion to the solid support. Densitometry of the gel confirmed that the amount of AB in lane 2 was negligible.

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