A fresh trim provides a new look at the human mannose receptor

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The human mannose receptor plays an important role in scavenging a variety of glycans and glycoconjugates, which contributes to both innate and adaptive immunity. However, the fine details of its ligand specificity, and specifically that of carbohydrate-recognition domain 4, the most functionally relevant C-type lectin domain within the receptor, are not completely understood. Feinberg *et al.* use glycan arrays, crystallography, and a newly trimmed version of carbohydrate-recognition domain 4 to elucidate the molecular mechanisms driving binding specificity. These data contribute to our molecular understanding of Ca^{2+}-mediated binding promiscuity in the human mannose receptor and the scavenging role of the receptor itself and highlight unexpected interactions that should inspire further study.

The type I transmembrane human mannose receptor CD206 is a C-type lectin found primarily on macrophages, immature dendritic cells, and liver sinusoidal endothelial cells. Its known role is to scavenge endogenous and foreign macromolecules through carbohydrate and protein recognition, and it is involved in both innate and adaptive immune responses. Ligands for the mannose receptor include endogenous glycoproteins, such as lysosomal enzymes, collagen, and pituitary hormones, as well as microbial surface glycan structures such as yeast mannans, mycobacterial mannose-capped lipoarabinomannans, and viral high-mannose oligosaccharides (1). However, the molecular determinants driving carbohydrate recognition by the mannose receptor for its multitude of diverse carbohydrate ligands are not fully understood. New work by Feinberg *et al.* (2) provides major advances on this front with high-resolution insight into the molecular determinants driving carbohydrate recognition across 15 crystal structures for one of the key carbohydrate-binding domains from this receptor.

The extracellular, ligand-binding portion of the mannose receptor is composed of three types of domains. The N-terminal domain is an R-type carbohydrate-recognition domain (CRD) that binds to nonreducing sulfo-*N*-acetyl-D-galactosamine moieties (3). The neighboring fibronectin type II domain binds to collagen fragments (4). This is followed by eight C-type lectin-like domains. Carbohydrate-recognition domain 4 (CRD4) and carbohydrate-recognition domain 5 account for most of the binding activity, and CRD4 is the only C-type lectin domain capable of independently binding glycans, suggesting that further investigations of CRD4 could shed light on mannose receptor recognition overall. C-type lectin domains such as CRD4 bind sugar ligands mediated through Ca^{2+} coordination in their primary carbohydrate-binding sites. CRD4 is known to bind terminal mannose, fucose, GlcNAc, and glucose residues (5). However, the only crystal structure of CRD4, published over 20 years ago, demonstrated an altered domain structure that prevented the capture of biologically relevant ligand complexes for crystallographic analyses (6).

To circumvent this problem, Feinberg *et al.* (2) first modified the mannose receptor domain to remove predicted flexible regions anticipated to enable domain swapping during crystallization. The resultant "trimmed CRD4" was purified on a mannose-Sepharose column, thus demonstrating carbohydrate-binding activity. The construct was further biotinylated and incubated with either streptavidin-coated plates for solid-phase competition binding assays or fluorescently-labeled streptavidin for screening on the Consortium for Functional Glycomics complex glycan array.

Glycan array screening revealed a fuller picture of the variety of glycans CRD4 is capable of binding. Nearly all the oligosaccharides giving a strong signal included a nonreducing D-mannose or L-fucose moiety (2). The identified D-mannose ligands are all substructures of a Man_9 high-mannose oligosaccharide, and the strongest D-mannose-related signals have terminal D-mannose-alpha-1,2-D-mannose motifs. The screen also revealed some unexpected results, such as binding of sulfated ligands and those that bear Lewis-a epitopes, but not those that contain Lewis-x.

A solid-phase competition assay allowed the authors to delve more deeply into the glycan interactions. The data showed a Man_9 oligosaccharide binds with 40-fold higher affinity than a D-mannose monosaccharide (2). Testing of disaccharides and trisaccharides suggested that avidity could be responsible for the tighter binding of the branched ligands. Indeed, the experimental setup of the assay concentrates CRD4 on the surface of streptavidin-coated plates, thus clustering the CRD4-binding sites together and providing a

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multivalent binding surface. A critical assessment to advance the understanding of the binding mechanism with branched ligands, and to distinguish between surface immobilization effects *versus* free CRD4, could be obtained by including a similar comparison using isothermal titration calorimetry. Future solution-based investigations of binding affinity will be important to determine whether these measurements are influenced by non-natural avidity and to provide a deeper understanding of the thermodynamic forces driving these biologically relevant protein–carbohydrate interactions.

Using X-ray crystallography, Feinberg *et al.* (2) examined the molecular interactions driving the specificity and promiscuity of CRD4 (Fig. 1). High-resolution crystal complexes unexpectedly demonstrate that the Ca^{2+}-coordinated glycans can interact in more than one orientation, even when binding the same monosaccharide in the primary binding site. D-mannose is coordinated in alternate orientations *via* its 3- and 4-OH groups (Fig. 1, *A*–*C*), methyl-*N*-acetyl-alpha-D-glucosamine is coordinated in alternate orientations *via* its 3- and 4-OH groups (Fig. 1, *D* and *E*), and L-fucose is coordinated *via* its 2- and 3-OH groups (Fig. 1, *F* and *G*) or in alternate orientations *via* its 3- and 4-OH groups (Fig. 1, *H*–*J*). Thus, this Ca^{2+}-mediated coordination is absolutely essential not only in CRD4 binding overall, as expected, but also in affording a binding site able to accommodate diverse carbohydrate ligands. How, then, does CRD4 maintain any specificity? The authors also provide insight into this question by identifying secondary interactions of CRD4 with complex carbohydrate ligands. Further investigation into more distal secondary interactions with relevant glycans would also contribute to our understanding on complex carbohydrate-binding specificity. Of particular interest for potential secondary interactions is a flexible, solvent-exposed hydrophobic residue (tyrosine 723) on a surface loop of CRD4 near the primary binding site, which seems poised to play a role in binding.

Untangling the mechanisms and driving forces of interactions and the full biological components of mannose receptor ligands remains a priority and further structure and function studies will be helpful; for example, further screening of microbial glycans could reveal unexplored host–pathogen interactions. Moreover, structural studies of the conformations of the extracellular domain of the mannose receptor have revealed it undergoes pH-dependent conformational changes, taking on an extended conformation at basic pH and a more condensed conformation at acidic pH (7–9). A tantalizing comparison of the extracellular multidomain mannose receptor components in solution demonstrating avid binding on immobilized multivalent ligands (10) provides a suggestion for the next frontier of molecular studies. Examination of conformational changes upon ligand binding, such as has already been exploited to a certain extent with the mannose receptor (8), along with biophysical characterization of binding interactions using a solution-based method, will be valuable in appreciating how avid binding contributes to mannose receptor structure and function.

Feinberg *et al.* have successfully demonstrated that even within only the one CRD4 domain, mannose receptor interaction with its carbohydrate ligands is extremely complex. This high-resolution structure/function study lends important new insights into the promiscuous power of Ca^{2+} coordination in biologically relevant protein–carbohydrate interactions. Furthermore, this contributes significantly toward our

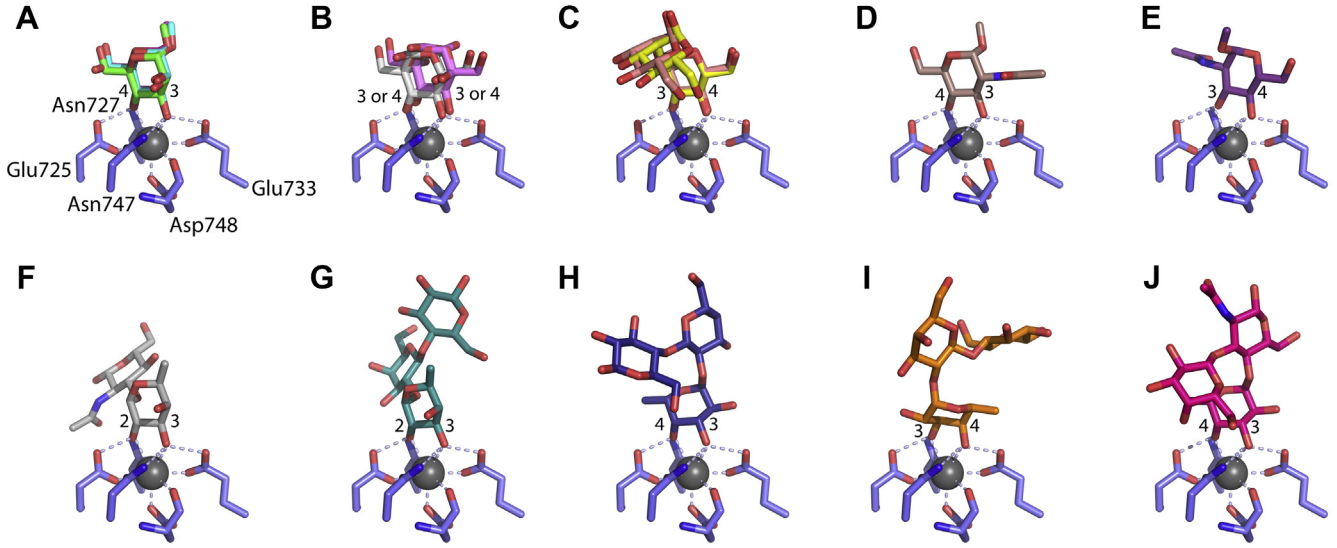

Figure 1. CRD4 primary monosaccharide-binding site interactions demonstrating the promiscuous power of Ca^{2+} coordination (2)**.** Carbons of the interacting monosaccharide hydroxyl groups are labeled adjacently for all the complexes. Ca^{2+} is shown as a *dark sphere*. Amino acids participating in the primary binding site are labeled in panel *A*. CRD4 is shown in complex with (*A*) methyl-alpha-D-mannose (three complexes from PDB IDL: 7JUB, 7JUC, and 7JUD), (*B*) D-mannose (*white*, dual occupancy, PDB ID: 7JUG) and D-mannose-alpha-1,6-D-mannose (*rose*, only the nonreducing dual occupancy monosaccharide could be modeled, PDB ID: 7JUH), (*C*) D-mannose-alpha-1,2-D-mannose (two complexes from PDB ID: 7JUE and 7JUF), (*D*) methyl-*N*-acetyl-alpha-D-glucosamine (PDB ID: 7L65), (*E*) methyl-*N*-acetyl-alpha-D-glucosamine (PDB ID: 7L66), (*F*) L-fucose-alpha-1,3-*N*-acetyl-D-glucosamine (PDB ID: 7L67), (*G*) L-fucose-alpha-1-2-D-galactose-beta-1-4-D-glucose (PDB ID: 7L63), (*H*) L-fucose-alpha-1-2-D-galactose-beta-1-4-D-glucose (PDB ID: 7L62), (*I*) L-fucose-alpha 1-2-D-galactose-beta-1-4-D-glucose (PDB ID: 7L61), and (*J*) the Lewis-A trisaccharide (PDB ID: 7L64). CRD4, carbohydrate-recognition domain 4.

molecular understanding of C-type lectin receptors, particularly the scavenging role of the mannose receptor and its mechanism of interaction with both endogenous and foreign macromolecules.

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Abbreviations—The abbreviations used are: CRD, carbohydrate-recognition domain; CRD4, carbohydrate-recognition domain 4.

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