Author(s): Suzanne J. Dilly, Andrew J. Clark, Daniel A. Mitchell, Andrew Marsh, Paul C. Taylor
Article Title: Using the Man9(GlcNAc)2 – DC-SIGN pairing to probe specificity in photochemical immobilization

Year of publication: Not yet published
Link to published article: None
Publisher statement: None

Using the $Man_9(GlcNAc)_2$ – DC-SIGN pairing to probe specificity in photochemical immobilization

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Received (in XXX, XXX) Xth XXXXXXXXX 200X, Accepted Xth XXXXXXXXX 200X
First published on the web Xth XXXXXXXXX 200X
DOI: 10.1039/b000000x

We demonstrate specificity of an immobilised oligosaccharide $Man_9(GlcNAc)_2$ upon a 96-well photochemical array, for its known receptor, the cell-surface lectin *D*endritic *C*ell–*S*pecific *I*CAM3 *G*rabbing *N*onintegrin (DC-SIGN).

Oligosaccharide surface display is used widely in medicinal chemistry discovery programmes,[1, 2] glycomic approaches to understanding function,[3] and associated biophysical measurements. Display methods often presume that a particular regiochemical point of attachment will lead to a desired bioactive partnership, however this may not always be the case,[4] potentially resulting in sub-optimal activity. In particular, a limited range of surface coupling chemistries are often utilized,[5, 6] which, compounded by the low availability of saccharide to be immobilized leads to real synthetic and biological challenges. The design of high-throughput assays for lectins has also been noted to be complicated by their tendency to bind weakly to monovalent carbohydrate ligands, hence methods for mimicking multivalency such as that found in the $Man_9GlcNAc_2$/DC-SIGN pairing continue to be developed.[7, 8] We here demonstrate that an array of photoactivatable chemistries **1**-**5**, **Figure 1**, in a standard 96-well format[9] allows rapid immobilisation of both mono- and oligosaccharides in a fashion that retains their anticipated biological activity. Recent work has shown the applicability of related surface-mediated photoactivation strategies[10] towards similar goals.[11, 12]

DC-SIGN is a human lectin primarily expressed on the surface of dendritic cells, and is known to interact with host glycoproteins of the immune system such as ICAM-3 *via* binding to selected glycans.[13] In addition, it also binds to carbohydrate structures on the surfaces of pathogens including HIV-1 and *Mycobacterium tuberculosis*. [14, 15]

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† Electronic Supplementary Information (ESI) available: immobilisation of saccharides and screening *versus* biotin-conjugated DC-SIGN. See DOI: 10.1039/b000000x/

Glycan array and radioligand competition assays have shown that soluble recombinant DC-SIGN binds selectively to branched *N*-linked high mannose oligosaccharides, notably $Man_9GlcNAc_2$, **7** (**Figure 2**, a component of the gp120 envelope glycoprotein of HIV-1) and also to fucosylated oligosaccharides such as Lewis-x and Blood Groups A and B.[16, 17]

In competition assays, monosaccharides such as mannose and fucose interact with DC-SIGN with K_I values between 0.6 and 1.0 mM, but in the case of $Man_9GlcNAc_2$, the K_I value is significantly lower at 16 μM, indicating higher affinity.[17] Crystallographic studies have shown that this enhanced affinity for selected oligomeric glycan structures is supported by extended binding surfaces of the lectin domain, allowing for multiple sites of protein-oligosaccharide contact.[18] However, our understanding of the extent of DC-SIGN ligand identity and mode of binding remains incomplete.

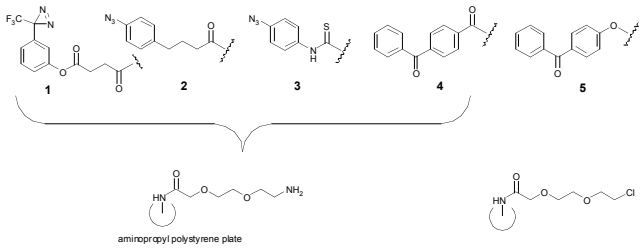

Figure 1 Photochemistries **1** – **5** on ethylene glycol linker derivatised polystyrene 96-well plates.

We have demonstrated that Magic Tag®, a chemical genomics tool developed in our laboratories[9, 19] can be applied to reveal potentially interesting interactions between small biologically active molecules and polypeptides from a phage displayed library representing a proteome. Of particular relevance is our earlier study in which we immobilised abscisic acid.[9] Extension of our easy-to-use array of photochemistries including a diazirine[10] **1**, two aryl azides[20] **2**, **3** and two benzophenones[20] **4**, **5** to explore binding events between saccharides and lectins is an appealing concept, but we had not previously validated Magic Tag® for this purpose. Herein, we demonstrate that the known specificities of DC-SIGN in solution is maintained towards three immobilized monosaccharides.

Results

Photochemical immobilization on surface tethered chemistries **1** – **5** together with Corning® photochemistry Universal-BIND™ of $Man_9GlcNAc_2$ (**Figure 2**), the known ligand for DC-SIGN revealed that diazirine **1** gave the strongest response when challenged with biotin-conjugated DC-SIGN and subsequently exposed to fluorescein isothiocyanate labelled anti-biotin (**Figure 3**). The same pattern of relative fluorescence was also seen when D-mannose, **6** (the ligand used in solid-phase to purify soluble recombinant DC-SIGN from bacterial cell extracts) was similarly immobilized and again exposed to DC-SIGN and streptavidin conjugates.

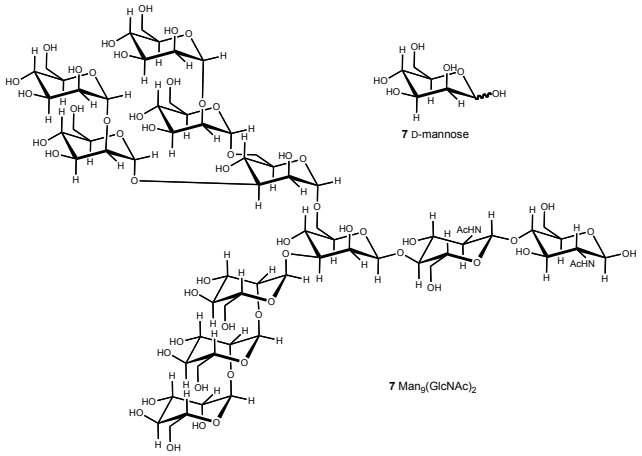

Figure 2 Structures of D-mannose **6** and a known ligand for DC-SIGN, $Man_9GlcNAc_2$ **7**.

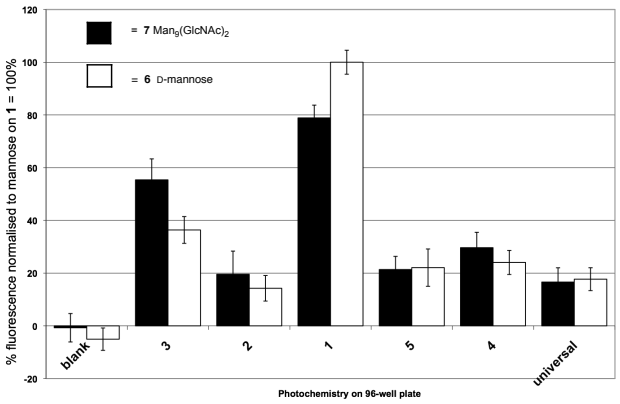

Figure 3 Response of derivatised surfaces **1** - **5**, blank reference and Universal-BIND™ to biotin-conjugated DC-SIGN probed with fluorescein isothiocyanate labelled anti-biotin.

In order to further explore the specificity of these novel readily prepared glyco-surfaces, two further monosaccharides, D-fucose and D-glucose (**Figure 4**) were immobilized using the same array of photochemistries (**Figure 5**). In turn, these surfaces gave lower fluorescent responses than D-mannose or the natural ligand, reflecting the known affinities of these monosaccharides (L-fucose 6.7±0.5 mM, D-mannose K_I = 13.1±0.4 mM and D-glucose, K_I = 23±1 mM respectively).[17] Note the difference in expected *vs*. our observed response for fucose may arise because we used D-fucose herein rather than L-fucose used in the earlier solution assay, both of which occur in nature.

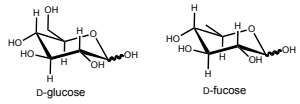

Figure 4 Structures of D-glucose and D-fucose.

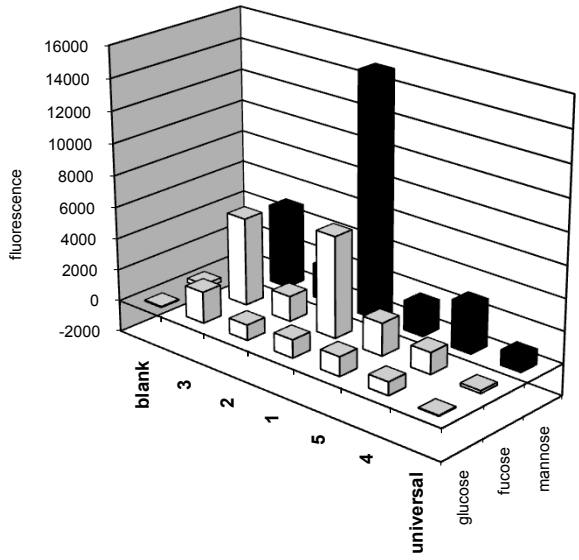

Figure 5 Comparison of D-glucose (*front*), D-fucose (*middle*) and D-mannose (*back*) immobilized on the photochemical array **1-5** and Universal-BIND™.

Overall, the results demonstrate that Magic Tag® has significant potential in the lectin field. The successful rapid immobilization of a complex natural ligand for DC-SIGN $Man_9(GlcNAc)_2$ that subsequently gave a positive response in our assay is very pleasing.

A second important observation is that the specific nature of the interaction between $Man_9GlcNAc_2$ and DC-SIGN (K_I = 16 μM)[17] appears to have been maintained. Both D-glucose and D-fucose, which are known to have lower binding constants with respect to DC-SIGN, gave the expected weak responses in our assay. On the other hand, D-mannose, which does bind to DC-SIGN, gave a significant response.

The data contain some interesting subtleties. Firstly, diazirine **1** shows greatest response to DC-SIGN when the known ligands $Man_9GlcNAc_2$, D-mannose and D-fucose are immobilized. 3-Trifluoromethyl-3-phenyl diazirine has previously been found to be a good photocrosslinker for DNA, with markedly different reactivity from both aryl azides and benzophenones. Our observations, taken in conjunction with

work to delineate surface *vs*. solution chemoselectivities[21] indicate the diazirine moiety to be especially useful in saccharide C-H or O-H bond insertions. Secondly, the responses to $Man_9GlcNAc_2$ and to mannose are closer than might have been expected from literature binding competition data.[17] This observation, while at first surprising, is in fact consistent with literature observations of surface density and avidity effects on immobilisation of saccharide ligands.[8] In our case, it may be that when the monosaccharide mannose is immobilized, clusters of the sugar result that mimic to some extent the natural ligand, $Man_9GlcNAc_2$, or that receptor oligomerization may be arising at the glycosylated surface. Indeed, the interaction between DC-SIGN and mannan, a natural oligomer of mannose, has been observed by 1D saturation transfer difference NMR[22] and surface-mediated DC-SIGN oligomerization itself has been postulated to play a key role in pathogen infectivity.[23]

Conclusions

The experiments described in this communication suggest that Magic Tag® photoimmobilisation is an efficient method for the capture of saccharides in order to probe their interactions with lectins. Molecular recognition of the immobilised sugars by DC-SIGN reproduced the known binding profile of this lectin, though mannose gave a surprisingly strong response, probably due to surface-mediated avidity effects. The use of a range of chemical functionalities in parallel enables rapid evaluation of conditions for photocapture and recognition of synthetically challenging small molecule ligands.

Acknowledgements

We thank Professor Richard Napier and Dr Andrew J. Thompson (Warwick HRI) for helpful discussions, BBSRC & EPSRC ExGen programme (SJD, ref. 88/EGM17690) and BBSRC Follow-on Fund (SJD, ref. BB/C524338/1) for funding. DAM is a Research Councils UK Academic Fellow.

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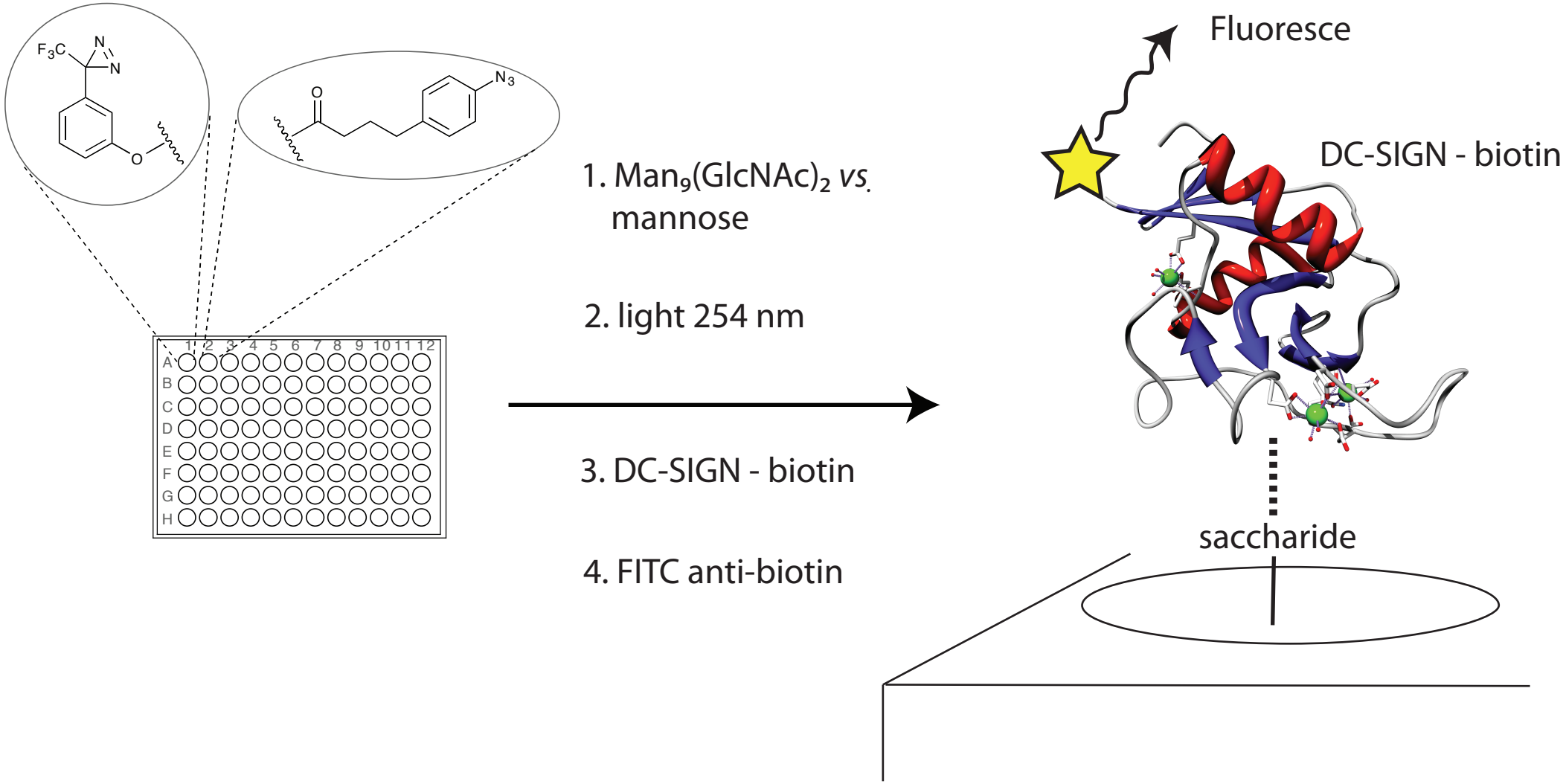
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1. Man9(GlcNAc)2 vs. mannose
2. light 254 nm
3. DC-SIGN - biotin
4. FITC anti-biotin
Fluoresce
DC-SIGN - biotin
saccharide