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

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Electronic Supporting Information

Experimental

Magic Tag® plates were prepared from Corning® Stripwell™ amine functionalized plates as described previously.[1] $Man_9GlcNAc_2$ was purified according to from soybean agglutinin as described previously[2] and dissolved in aqueous buffer to give a stock solution (1% CHES; 1.7 mg / ml). Soluble recombinant DC-SIGN extracellular domain was purified as described previously,[2] labelled with biotin by reaction with *N*-hydroxysuccinimidyl biotin (Pierce Chemical Co.), repurified on mannose-sepharose, and held as a stock solution in 1% *N*-cyclohexyl-2-aminoethanesulfonic acid buffer at *p*H 8.6 (1% CHES buffer; 1 mg / ml). Streptavidin-fluorescein isothiocyanate (FITC) conjugate was purchased from Sigma and used at 1 in 250 dilution of a 1 mg / ml 1% CHES buffer at *p*H 8.6 solution. D-Mannose, D-glucose and D-fucose were purchased from Aldrich and used as received.

Magic Tag® plate wells bearing chemistries **1**-**5**, OEG-NH_2 linker and Corning Universal-BIND™ respectively were treated in triplicate with $Man_9(GlcNAc)_2$ (50 μl / well of the above stock solution diluted to 5.7 μg / ml), D-mannose (50 μl / well of a 0.1 mg / ml solution) or water (50 μl / well) and irradiated under a handheld 254 nm source for 16 h. After this time, the wells were emptied, then irradiated for a further 10 min before a blocking solution of 2% bovine serum albumin solution (0.2 ml / well) was added and the plate incubated for 1h at r.t. The wells were again emptied and then treated with DC-SIGN biotin conjugate (50 μl / well of the above stock solution diluted 1:500 to give 5 μg / ml working solution) and incubated for 1 h at r.t. The wells were treated with streptavidin-FITC solution (50 μl / well) and incubated for 1 h, then washed six-fold with 0.5% CHES buffer at pH 8.6 (0.2 ml / well). The fluorescence (λ_{ex} = 485 nm; λ_{em} = 520 nm) was recorded on a Tecan GENios plate reader. An average was taken of the fluorescence reading from each triplicate segment containing $Man_9(GlcNAc)_2$ or D-mannose and the corresponding value from the wells containing water only was subtracted. The data displayed in Figure 3 is normalized to show observed fluorescence against wells containing D-mannose at 100% with error bars representing the standard deviation for triplicate measurements.

The above procedure was repeated using D-mannose, D-glucose, D-fucose and water in quadruplicate for each chemistry **1**-**5** and duplicate for other wells.

Tag	**$Man_9(GlcNAc)_2$**			**mannose**			**control** (water)	
1	31647	31087	30814	32940	32691	33472	23750	24783
2	26938	27742	28482	26793	27628	27346	25823	26191
3	31723	31631	32882	30823	29938	30473	26896	27550
4	27099	28080	27840	27148	27597	26799	25144	25003
5	27208	27844	28047	28427	27664	27198	25738	25916
Universal	26490	27438	27015	27252	27339	26641	25281	25769

Table 1a shows **Figure 3** raw data from experiment in triplicate probing immobilised ligand with DC-SIGN-biotin followed by FITC-labelled streptavidin. Fluorescence (arbitrary units) is shown for each Magic Tag® chemistry **1-5** and Corning® Universal-BIND™ with (left to right) $Man_9(GlcNAc)_2$, (50 μl / well of 5.7 μg / ml solution) D-mannose (50 μl / well of a 0.1 mg / ml solution) or water (50 μl / well).

Tag	Mean			Standard Deviation		
	$Man_9(GlcNAc)_2$	**mannose**	**water**	**$Man_9(GlcNAc)_2$**	**mannose**	**water**
1	31183	33034	24267	425	399	730
2	27721	27256	26007	772	425	260
3	32079	30411	27223	697	446	462
4	27673	27181	25074	511	400	100
5	27700	27763	25827	438	620	126
Universal	26981	27077	25525	475	380	345

Table 1b shows **Table 1a** and **Figure 3** arithmetic means and standard deviations (S.D.).

Tag	**Mean Tag – mean control**	
	$Man_9(GlcNAc)_2$	mannose
1	6916	8768
2	1714	1249
3	4856	3188
4	2600	2108
5	1873	1936
Universal	1456	1552

Table 1c shows mean fluorescence for each ligands immobilised on Magic Tag® chemistries **1** – **5** with fluorescence for wells containing water (control) subtracted, as depicted in **Figure 3**.

Tag	**mannose**		**fucose**		**glucose**		**control** (water)	
1	36694	35006	28169	26936	22121	21042	20119	21003
	36835	35831	26969	27130	22616	22586	21827	20876
2	25035	25593	24340	24440	23655	23715	22705	23295
	25695	24964	24874	25300	24433	24683	23030	23609
3	28581	28425	29846	28905	25781	25124	22671	23770
	28253	27963	28716	27902	26103	24219	23120	23793
4	23818	23735	23300	23664	22643	21669	21336	21522
	25532	24972	21928	22663	22942	22239	22060	21248
5	24688	24222	24627	24625	23100	23863	22577	22236
	23754	23143	23259	24022	23665	22601	22209	21360
Universal	22640	22969	21837	22227	21672	21968	21554	22169

Table 2a: shows raw fluorescence data for **Figure 5** from experiment in triplicate probing immobilised ligand with DC-SIGN-biotin followed by FITC-labelled streptavidin. Fluorescence (arbitrary units) is shown for each Magic Tag® chemistry **1-5** and Corning® Universal-BIND™ with (left to right) D-mannose, D-glucose, D-fucose (50 ml / well of a 0.1 mg / ml solution) and water (50 ml / well).

Tag	Mean				Standard Deviation			
	Mannose	**Fucose**	**Glucose**	**Water**	**Mannose**	**Fucose**	**Glucose**	**Water**
1	36092	27301	22091	20956	849	585	735	700
2	25322	24739	24122	23160	376	440	515	385
3	28306	28842	25307	23339	265	798	832	544
4	24514	22889	22373	21542	883	762	551	364
5	23952	24133	23307	22096	660	649	571	518
Universal	22805	22032	21820	21862	233	276	209	435

Table 2b shows **Table 2a** and **Figure 5** means and standard deviations.

Tag	Mean Tag – Mean water		
	Mannose	**Fucose**	**Glucose**
1	15135	6345	1135
2	2162	1579	962
3	4967	5504	1968
4	2973	1347	832
5	1856	2038	1212

Table 2c shows mean fluorescence for each saccharide immobilised on Magic Tag® chemistries **1** – **5** with fluorescence for wells containing water (control) subtracted, as depicted in **Figure 5**.

Additional references and discussion

The photochemistries featured herein have been used for a variety of applications, some of which are reviewed by Fleming[3] and the references as indicated in the main text. The functionality chosen included a diazirine[4-11] **1**, two aryl azides[12, 13] **2**, **3** and two benzophenones[14, 15] **4**, **5**.

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